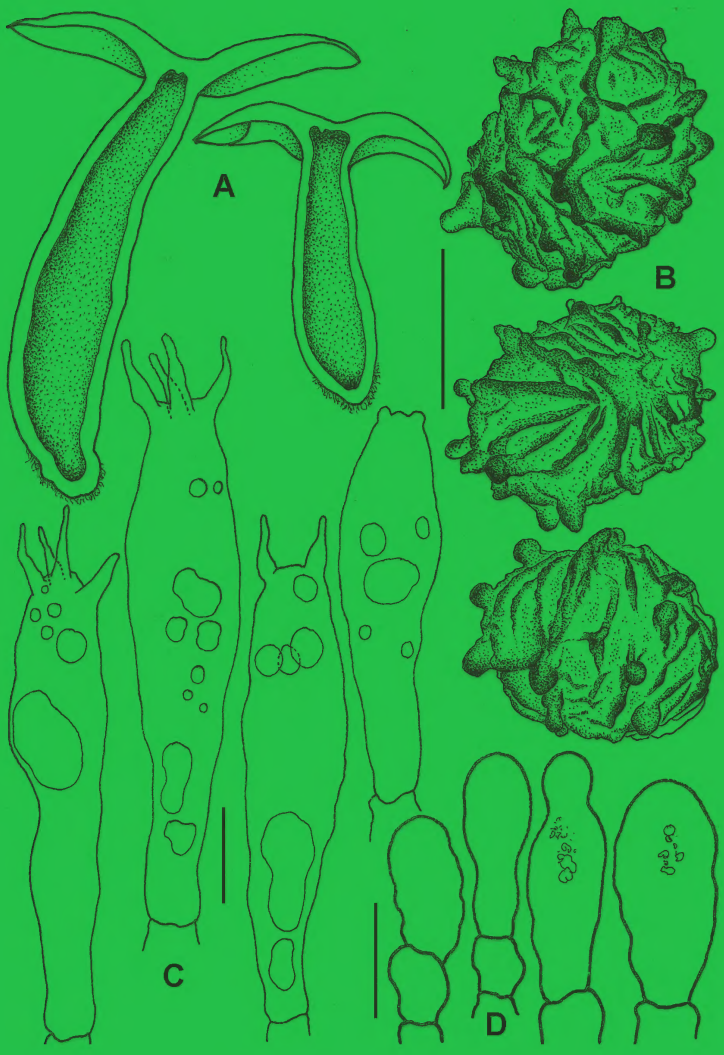
MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 130(1) JANUARY–MARCH 2015



Lactarius olivaceoglutinus sp. nov.
(Das, Verbeken & Nuytinck— PLATE 4, p. #115)
KANAD DAS, *artist*

ISSN (PRINT) 0093-4666 http://dx.doi.org/10.5248/130 ISSN (ONLINE) 2154-8889
MYXNAE 130(1): 1–306 (2015)

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Published by
Mycotaxon, Ltd.
p.o. box 264, Ithaca, NY 14581-0264, USA
www.mycotaxon.com & www.ingentaconnect.com/content/mtax/mt
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MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 130(1)

JANUARY–MARCH 2015

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CONSISTING OF I–X + 306 PAGES INCLUDING FIGURES

ISSN 0093-4666 (PRINT) http://dx.doi.org/10.5248/130(1).cvr ISSN 2154-8889 (ONLINE)

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MYCOTAXON

VOLUME ONE HUNDRED THIRTY (1) — TABLE OF CONTENTS

Cover section

Errata vi
Reviewers vii
Submission procedures viii
From the Editor ix

Research articles

Pseudoplectania lignicola sp. nov. described from central Europe
S. Glejdura, V. Kučera, P. Lizoň, & V. Kunca 1

Additions to rust and chytrid pathogens of Turkey
Cumali Özaslan, Makbule Erdoğdu, Elşad Hüseyin, & Zekiye Suluder 11

Dentipellicula austroafricana sp. nov. supported by morphological and phylogenetic analyses Jia-Jia Chen, Lu-Lu Shen, & Yu-Cheng Dai 17

A new species of *Terriera* (*Rhytismatales, Ascomycota*) on *Photinia villosa* Qing Li, Yuan Wu, Dan-Dan Lu, Ya-Fei Xu, & Ying-Ren Lin 27

New records of one *Amygdalaria* and three *Porpidia* species (*Lecideaceae*) from China Lu-Lu Zhang, Xin Zhao & Ling Hu 33

Anungitea guangxiensis and *Ellisembia longchiensis,* two new species from southern China Ji-Wen Xia, Ying-Rui Ma, & Xiu-Guo Zhang 41

Fistulina subhepatica sp. nov. from China inferred from morphological and sequence analyses
Jie Song, Mei-Ling Han, & Bao-Kai Cui 47

Antherospora sukhomlyniae, a new species of smut fungi on *Hyacinthella* in Crimea (Ukraine) Kyrylo G. Savchenko 57

Tuber xanthomonosporum, a new *Paradoxa*-like species from China
Yuan Qing, Shu-hong Li, Cheng-yi Liu, Lin Li, Mei Yang, Xiao-lei Zhang, Xiao-lin Li , Lin-Yong Zheng, & Yun Wang 61

Distance1D – a protein profile analytical program designed for fungal taxonomy
Dušan Materić, Biljana Kukavica , & Jelena Vukojević 69

Coccomyces prominens sp. nov. (*Rhytismataceae*) on *Rhododendron coeloneurum* in China Ya-Fei Xu, Yuan Wu, Yan-Qiong Meng, Shi-Juan Wang, & Ying-Ren Lin 73

Type studies on *Amauroderma* species described by J.D. Zhao et al. and the phylogeny of species in China Meng-Jie Li & Hai-Sheng Yuan 79

Cladonia corymbescens consists of two species
Teuvo Ahti, Raquel Pino-Bodas, & Soili Stenroos 91

Morphology and phylogeny of four new *Lactarius* species from Himalayan India
Kanad Das, Annemieke Verbeken, & Jorinde Nuytinck 105

Synonymy of two species of *Bipolaris* from aquatic crops of *Poaceae*
Zi-Lan Xiao, Kevin D. Hyde, & Jing-Ze Zhang 131

Myxomycetes of Sonora (Mexico) 6. Central plains of the Sonoran Desert
Marcos Lizárraga, Gabriel Moreno, Martín Esqueda, Cynthia Salazar-Márquez, & Martha L. Coronado 145

New taxa of *Ambomucor* (*Mucorales*, *Mucoromycotina*) from China
Xiao-Yong Liu & Ru-Yong Zheng 165

Perenniporia koreana, a new wood-rotting basidiomycete from South Korea
Yeongseon Jang, Seokyoon Jang, Young Woon Lim, Changmu Kim, & Jae-Jin Kim 173

New records of corticolous myxomycetes from Turkey
R. Batur Oran & C. Cem Ergül 181

A new species of *Lophodermium* with variously branched paraphyses
Hai-Lin Gu, Ya-Fei Xu, Dan-Dan Lu, Shi-Juan Wang, & Ying-Ren Lin 191

Bertia hainanensis sp. nov. (*Coronophorales*) from southern China
Larissa N. Vasilyeva, Hai-Xia Ma, Aleksey V. Chernyshev, & Steven L. Stephenson 197

Distribution of *Alternaria* species among sections. 1. Section *Porri*
Philipp B. Gannibal 207

Morchella galilaea, an autumn morel from Turkey
Hatira Taşkin, Hasan Hüseyİn Doğan, & Saadet Büyükalaca 215

Hyphoderma hallenbergii, a new corticioid species from India
Maninder Kaur, Avneet P. Singh, & G.S. Dhingra 223

Xylaria thailandica– a new species from southern Thailand
Natthapach Srihanant, Vasun Petcharat, & Larissa N. Vasilyeva 227

Rosellinia brunneola sp. nov. and *R. beccariana* new to China
Wei Li & Lin Guo 233

Phyllachora hainanensis sp. nov. from China
Na Liu, Le Wang, Gu Huang, & Lin Guo 237

Two new species of *Zasmidium* from Nepal
Ravindra Nath Kharwar, Archana Singh, Raghvendra Singh, & Shambhu Kumar 241

The lichen genus *Leiorreuma* in China
Xiao-Hua Wang, Li-Li Xu, & Ze-Feng Jia 247

Periconiella liquidambaricola sp. nov. – a new Chinese hyphomycete
Uwe Braun, Steffen Bien, Lydia Hönig, & Bettina Heuchert 253
First record of *Erysiphe syringae-japonicae* in Turkey
Ilgaz Akata & Vasyl P. Heluta 259
AFLP characterization of three argentine *Coprotus* species
Araceli Marcela Ramos, Luis Franco Tadic,
Nahuel Policelli, Laura Ines Ferreyra, & Isabel Esther Cinto 265
Verticicladus hainanensis, a new aquatic hyphomycete
Ming-Tian Guo, Min Qiao, Jian-Ying Li, Wei Wang, & Ze-Fen Yu 275
Geographic distribution of *Sarcoporia polyspora* and
Sarcoporia longitubulata sp. nov.
Josef Vlasák, Josef Vlasák Jr., Juha Kinnunen, & Viacheslav Spirin 279
Coccomyces neolitseae sp. nov. from Zhangjiajie, China
Qing Li, Yu-Xia Chen, Chun-Tao Zheng, Dan-Dan Lu, & Ying-Ren Lin 289
Datronia ustulatiligna sp. nov. (*Agaricomycetes*) from India
Harpreet Kaur, Gurpreet Kaur, & G.S. Dhingra 295
Three new species of *Xylaria* from China
Gu Huang, Ruisha Wang, Lin Guo, & Na Liu 299
Nomenclatural novelties and typifications
proposed in Mycotaxon 130-1 305

Errata from previous volumes

Volume 129

p.293, line 2	for: Hasan Hüseyin Doğan	read: Hasan Hüseyİn Doğan
p.296, line 32	for: Lado, C.	read: Lado C.
p.455, line 28	for: DM100	read: DM1000
p.457, line 3	for: Spores ornamented with long ridges, arranged in an incomplete banded reticulum.	read: Spores were decorated with long ridges sometimes forming an incomplete banded reticulum.
p.457, line 17	for: (ca. 9–12 µm diam.) and unbranched and thicker	read: (ca. 9–12 µm diam.) and thicker
p.458, line 9	for: National Basic Research Program of China (3140010180)	read: National Basic Research Program of China (3140010180) and National Science and Technology Foundation Project (2014FY210400)

Reviewers — volume one hundred thirty (1)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

André Aptroot
N.S. Atri
Z.M. Azbukina
Gerald L. Benny
Lina Bettucci
Uwe Braun
Ana Rosa Burgaz
Lei Cai
Matteo Carbone
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Xiang-Hua Wang
Zheng Wang
A.J.S. Whalley
Ming Ye
Alessandra Zambonelli
Xiu-Guo Zhang
Li-Wei Zhou

publication date for volume one hundred twenty-nine (2)
MYCOTAXON *for* October–December 2014, (i–vi + 215–495)
was issued on January 8, 2015

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From the Editor-in-Chief

Welcome to the year of Mycotaxon 130! — For several decades we have determined Mycotaxon volume size by the number of pages (~512/volume). In recent years, however, our condensed format has — for most papers — produced pages containing far more words than those found in our older volumes, so that it has taken longer to fill a volume.

Beginning with Mycotaxon 130, we move to the publication of one volume (issued quarterly) per year. Pagination will run consecutively through all four issues, but we will continue to list expert reviewers, errata, and nomenclatural novelties in each quarterly for immediate reference. Although the subscription period now coincides with the calendar year, those subscribing to our journal only for 2015 will still be able to access Mycotaxon 130 papers in future years for no extra access fee. Our new 'nomenclatural' change brings us in line with other scholarly journals, most of which also assess annual subscription rates per 'volume.'

We are pleased to announce that with this move, we are finally back on schedule. The 2015 January–March quarterly (this issue) presents all papers submitted to the *Editor-in-Chief* as of March 31. No longer tied to a targeted number of pages per volume, we will be able to deliver future quarterlies promptly and on time. Librarians (and authors) rejoice!

Mycotaxon 130(1) contains 37 research papers by 132 authors (representing 19 countries) and revised by 63 expert reviewers.

Within its pages are 32 species and one variety new to science representing *Ambomucor, Anungitea, Bertia, Coccomyces, Ellisembia, Fistulina, Leiorreuma, Lophodermium, Periconiella, Phyllachora, Rosellinia, Terriera, Tuber, Verticicladus,* and *Xylaria* from China; *Datronia, Hyphoderma,* and *Lactarius* from India; *Dentipellicula* from South Africa; *Perenniporia* from Korea; *Pseudoplectania* from Slovakia and the Czech Republic; *Sarcoporia* from the Canary Islands and the U.S.A.; *Xylaria* (again) from Thailand, and *Zasmidium* from Nepal. We also offer one new name in *Periconiella,* one new *Cladonia* combination (resulting from the taxonomic clarification of *C. corymbescens*), and phylogenetic and pathogenicity confirmation of the synonymy of *Bipolaris zizaniae* with *B. oryzae.*

In addition to range extensions for previously named taxa of myxomycetes (for Turkey and Mexico) and chytrids, mildews, morels, and rusts (for Turkey), we present a type study of *Amauroderma* species from China, a key to Chinese *Leiorreuma* species, and a complete list of *Alternaria* species assigned to *A.* sect. *Porri.* The applicability of Distance 1D protein profile analysis for fungi and AFLP analyses in *Coprotus* are also evaluated.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
13 April 2015

ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.1

Volume 130, pp. 1–10 *January–March 2015*

Pseudoplectania lignicola sp. nov. described from central Europe

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Abstract — A new species from Slovakia and the Czech Republic, *Pseudoplectania lignicola,* is described and illustrated. It is distinguished from other members of the genus by a centrally arranged globose membranous sheath surrounding the spores, thick ectal excipulum of oblong cells at the apothecial base, and growth on less specific biotopes. Comparisons with similar species and the diagnostic significance of membranous sheath surrounding the ascospores are also discussed.

Keywords — biodiversity, *Sarcosomataceae*, typification

Introduction

The genus *Pseudoplectania* was established by Fuckel (1870) for two fungi, *Peziza nigrella* Pers. and *Peziza fulgens* Pers. *Pseudoplectania nigrella* was selected by Seaver (1913) as the lectotype of the genus, and the species itself was neotypified by Carbone & Agnello (2012). *Peziza fulgens* is today treated in the genus *Caloscypha* Boud.

Eleven species are currently accepted in the genus: *Pseudoplectania affinis*, *P. carranzae*, *P. ericae*, *P. kumaonensis*, *P. melaena, P. nigrella*, *P. ryvardenii*, *P. sphagnophila*, *P. stygia*, *P. tasmanica*, and *P. vogesiaca* (Carbone et al. 2013, 2014; Iturriaga et al. 2012).

The first finding of the new *Pseudoplectania* reported here was associated with *Leucobryum glaucum* and tentatively identified in situ as *P. sphagnophila.* The new fungus was later collected on *Abies alba* wood buried in soil as well as among *Sphagnum* bogs. The species was mostly collected on conifer wood.

Materials & methods

The study is based on the morphological examination of 11 collections from three localities in Slovakia and one in the Czech Republic. The macro-morphological characters were observed in fresh material. The micro-morphological structures were studied in fresh and dried material using a light microscope with oil immersion lens (×1000). Vertical sections of material were examined in 5% KOH, tap-water, Melzer's reagent, and a Congo Red + ammonia solution. Values of micro-morphological characters were estimated as average plus and minus a standard deviation of 30 measurements for each taxon (with 10 and 90 percentiles presented in parentheses). All descriptions are based on studied specimens. Localities are georeferenced and the coordinates are in WGS 84 system. Examined specimens are kept in herbarium BRA, SAV, and HR. The abbreviations of herbaria are cited in accordance with Index Herbariorum (Thiers 2014).

Taxonomy

Pseudoplectania lignicola Glejdura, V. Kučera, Lizoň, Kunca, **sp. nov.** FIGS 1, 2

MycoBank MB 805097

Differs from *Pseudoplectania nigrella* by ascospores surrounded by a centrally arranged globose membranous sheath and by elongated cells in the outer ectal excipulum layer forming a palisade perpendicular to the surface.

TYPE: Slovakia, Nízke Tatry Mts., ca. 3 km N of Hiadeľ village, 48°50′7.38″N 19°18′39.84″E, alt. 700 m, SW exposure, 20° slope, dystric cambisol, biotite granodiorite, in a 100-year old managed forest: *Picea abies* (L.) H. Karst. 77%, *Abies alba* Mill., 17%, *Larix decidua* Mill. 6%; on wood of *Abies alba*, 21.IV.2010, leg. S. Glejdura (**Holotype**, BRA CR-19347).

ETYMOLOGY: *lignicola* (Latin) = living on wood. Most specimens were collected from wood.

APOTHECIA at first hemispherical, later cup shaped, plane at full maturity, 1–2.7 cm diam, substipitate or sessile. Hymenium smooth, dark brown to blackish brown. Margin composed of thick-walled cylindrical or slightly clavate hairs 15–110 µm long, 5.5–6.1 µm broad, with 1–3 septa and 1.5–2.3 µm thick walls. Receptacle densely tomentose, covered with thick-walled flexuose, coiled, distantly septate (60–185 µm between septa), black-brown to black hairs, ≤600 µm × 4.6–5.5 µm, walls 0.9–1.5 µm thick.

ECTAL EXCIPULUM 180–200 µm thick, outermost zone 2–4 cells thick, cells dark brown, thick-walled, subglobose to elongated 20–27 × 14–18 µm, inner layer of subhyaline elongated cells forming a palisade perpendicularly oriented to the surface, cells 34–92 × 15–29 µm.

MEDULLARY EXCIPULUM embedded in a gelatinous matrix, outer layer a 180–200 µm thick hyaline textura intricata, cells cylindrical, allantoid, narrowly pyriform 40–60 × 12–22 µm. Inner layer a 500–600 µm thick hyaline densely packed textura intricata, hyphae 9–13 µm diam. SUBHYMENIUM 30–40 µm

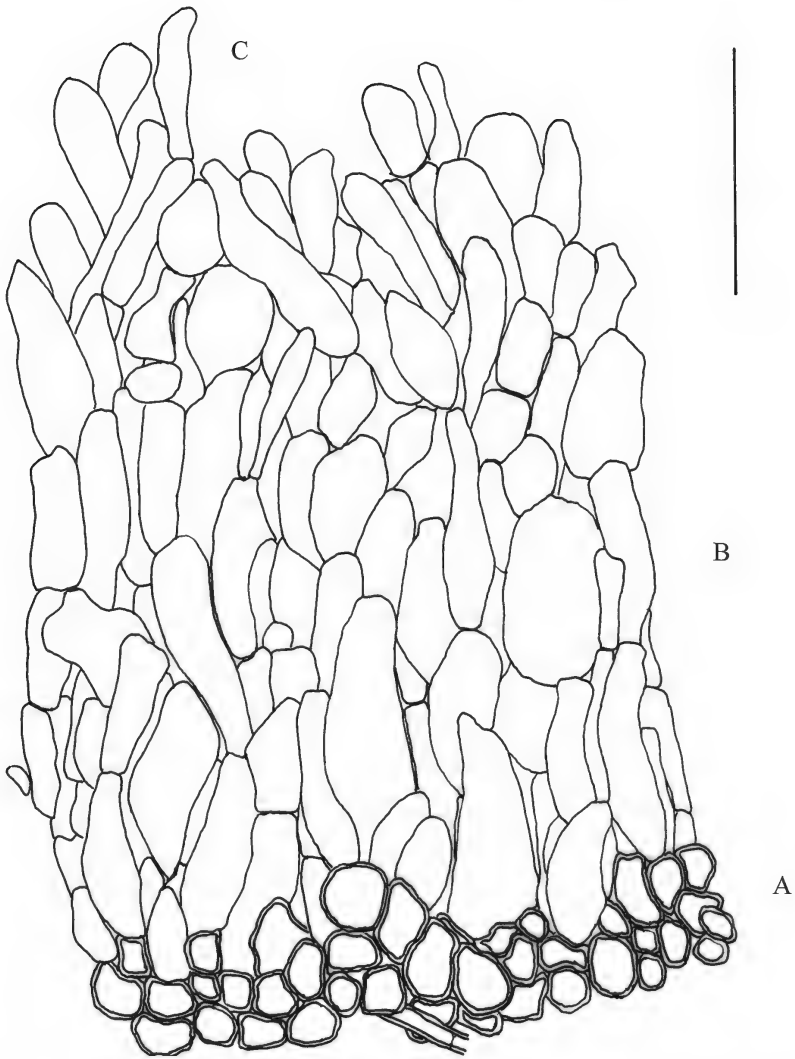

FIGURE 1. *Pseudoplectania lignicola* (Holotype BRA CR-19347): **A.** Ectal excipulum, outermost thick-walled cells. **B.** Inner layer of the ectal excipulum, elongated cells perpendicularly arranged to the surface. **C.** Distantly arranged cells of medullary excipulum. Scale bar = 50 μm.

thick, composed of light brown densely arranged cylindric, globose and ellipsoid cellular hyphae 3–4.6 μm diam.

ASCI narrowly cylindrical, tapered below to thin flexuous aporhynchous base (simple-septate), apex J–, operculate, 8-spored, 259–299 × 12.3–13.7 μm.

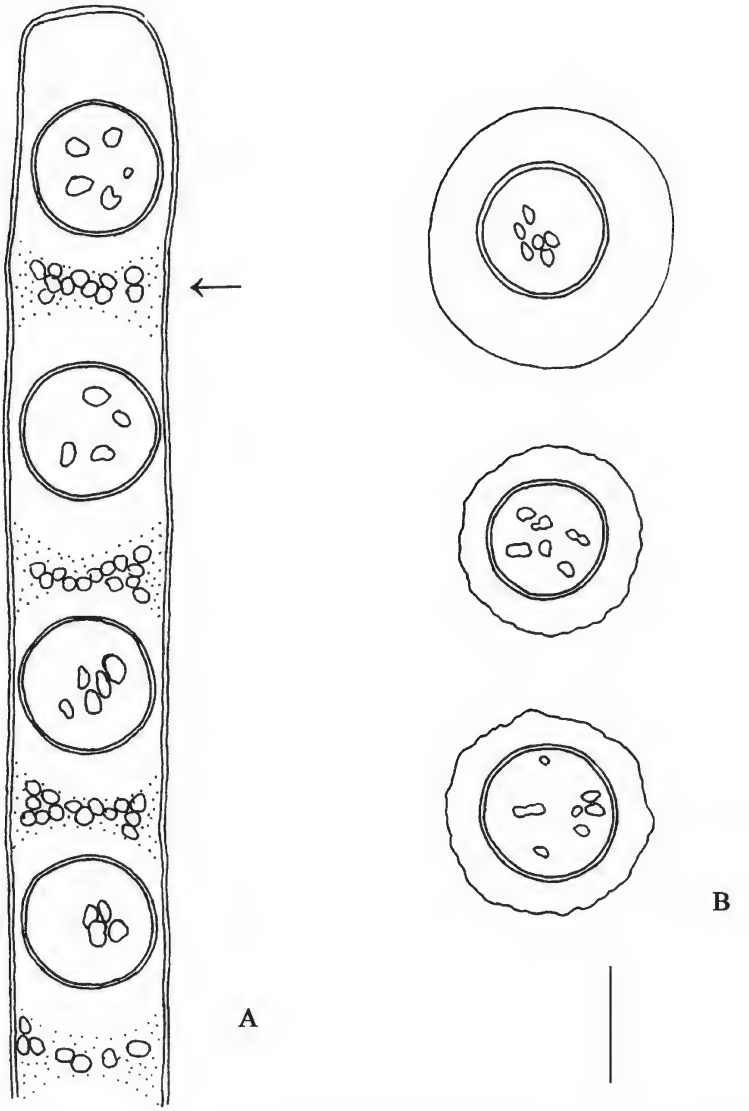

FIGURE 2. *Pseudoplectania lignicola* (Holotype BRA CR-19347). **A.** Ascus with ascospores from living apothecium; cytoplasmic granules concentrated symmetrically in a limited space by membranaceous sheath (arrowed). **B.** Ascospores with centrally arranged membranaceous sheath, from living apothecium. Scale bar = 10 µm.

ASCOSPORES (mature) globose, thick-walled, with granulate or small guttulate non-oleaginous content (negative reaction in SUDAN 4), (10.2–)11–12.2(–12.3)

µm. Mature ascospores surrounded by a membranous cover (sheath) 15.5–19.5 µm (including the sheath). The spores are visible in the center of the sheath in almost all mature ascospores, and in exsiccatae sheath remnants are at least visible on several spores. Ascospores within membranous sheaths are separated by cytoplasmic granules laid crosswise in the ascus. Spore walls usually swollen in 5% KOH, with the membranous sheaths dissolving in the same medium.

Paraphyses cylindric, septate, brownish in the upper part and often agglutinated with an amorphous substance, sometimes branched and anastomosed in basal and medial parts, 2.2–2.8 µm diam, apex mostly straight, often lobed or diverticulate, with lateral proliferations in upper part, enlarged to 2.8–3.6 µm diam. Irregularly cylindrical paraphyses can be observed in young or senescent apothecia. Light brown aseptate paraphyses with slightly thicker walls (so-called hymenial hairs) also present in all stages of apothecial development.

Ecology & Distribution: The species is known from Slovakia and the Czech Republic and possibly also from Norway (Eckblad 1968, see comment below). All available collections come from sub-montane and montane moist coniferous forests (dominated by *Picea abies)* on acid soils.

Additional specimens examined: **SLOVAKIA**, **Nízke Tatry Mts.**, ca. 3 km N of Hiadeľ village, buffer-zone of Nízke Tatry National Park, 48°50′7.38″N 19°18′39.84″E, alt. 700 m, in the forest, SW exposition, 20° slope, sporadically rocky, dystric cambisol, biotite granodiorite, two-storeyed 100-year old production forest, 1st storey: *Picea abies* 77%, *Abies alba* 17%, *Larix decidua* 6%; 2nd storey: *Fagus sylvatica* L. 100%, among moss *Leucobryum glaucum* Schimp., 20.IV.2008, leg. P. Štefanovie (SAV F-10755); on buried wood of *Picea abies*, 23.III.2010, leg. S. Glejdura (SAV F-10756); on wood of *P. abies*, 29.V.2011, leg. S. Glejdura (SAV F-10757); **Veporské vrchy Mts.**, ca. 1 km SW of Sihla village, Nature Reserve Vrchslatina, 48°39′18.96″N 19°37′58.50″E, alt. 900 m, in the forest, flat, 0° slope, dystric histosol (organic soil), diluvial organic sediments (upland moor) on biotite tonalites and granodiorites, three-storeyed ca 65-year old protective forest, 1st storey: *Picea abies* 100%, 2nd, 3rd storey: *Picea abies* 80–90 %, *Alnus incana* Mill. 20–10%, *Sphagno palustris-Piceetum*, well-preserved wetland to peatbog biocenoses with occurrence of specific ecotype of spruce (resonance wood), on buried wood of *P. abies*, 28.V.2010, leg. S. Glejdura & V. Kunca (SAV F-10758); on buried wood of *P. abies*, leg. S. Glejdura & V. Kunca (SAV F-10759); 2.VI.2010 (SAV F-10760); **Vysoké Tatry Mts.**, Tichá dolina valley, ca. 6 km NNE of Podbanské village, National Nature Reserve Tichá dolina, 49°12′1.20″N 19°55′33.24″E, alt. 1140 m, in the forest, NW exposition, 35° slope, typical podzol soil, diluvial sediments on porphyric granitoids and granites, one-storeyed 55-year old protective forest, *Picea abies* 65%, *Sorbus aucuparia* L. 35%, on dead *Sphagnum* sp. and needles of *P. abies*, 18.V.2011, leg. S. Glejdura (SAV F-10761); on buried wood of *Picea abies*, among *Sphagnum* sp. bog, 18.V.2011, leg. S. Glejdura (SAV F-10762); on cone of *Picea abies*, 18.V.2011, leg. S. Glejdura (SAV F-10763).

CZECH REPUBLIC, **Hradec Králové**, Orlická tabule, Chvojnická plošina, 50°10′45″N 15°54′15″E, alt. 260 m, among moss *Leucobryum glaucum* in mixed forest of *Picea abies* and *Pinus sylvestris*, 1.IV.2011, leg. Z. Egertová & M. Sochor (HR 89756).

Discussion

Pseudoplectania is a morphologically and genetically well-delimited genus in the *Sarcosomataceae* Kobayasi (Carbone et al. 2013, 2014). Identification of taxa is based on the paraphysis morphology, ascospore and apothecium size, and substrate preferences. As we have presented in this paper, another character—the shape of the gelatinous (mucilaginous) sheath of the spore—must also be considered an important diagnostic character. Its nature distinguishes similar species: *P. nigrella*, *P. melaena*, and *P. lignicola*. It is easily detected in fresh material, less in dry herbarium specimens, although in the type specimen of *P. lignicola* the gelatinous sheath was still visible as late as three years after collection. Spores of *P. lignicola* lie at the centre of a spherical sheath, whereas in *P. nigrella* spores lie to one side of the surrounding sheath (Figs 2, 3). The hemispherical sheath of spores of *P. melaena* does not surround the spores but lies laterally adjacent to the spore surface (Fig 4), a phenomenon that has not been reported before.

Aside from the spore sheath morphology, *Pseudoplectania nigrella* (Pers.) Fuckel differs from *P. lignicola* by the thickness and textura of the ectal excipulum as well as by habitat and substrate preferences (on soil in coniferous forests, rarely on wooden substrates). Also, the ectal excipulum at the base of its apothecium is thinner (80–100 µm) and composed of smaller cells (17–25 µm diam) having a textura angularis–globulosa. Eckblad (1968: 119, fig. 65d) illustrated a specimen of *P. nigrella* from Norway that has centrally positioned spores in a membranous sheath. We suspect that this could well represent *P. lignicola,* so that our new species may have a wider distribution than currently recorded.

Pseudoplectania melaena (Fr.) Sacc., which has bigger (20–60 mm diam) stipitate apothecia and apically hooked paraphyses, grows on *Abies alba* branches or logs buried in wet soil. *Pseudoplectania sphagnophila* (Pers.) Kreisel, associated with *Sphagnum* sp., has smaller apothecia (10–15 mm diam), and its paraphyses, which may or may not have lateral branches on the upper parts, are apically hooked, as in *Otidea* (Kreisel 1962, Bauer 1999). *Pseudoplectania ericae* Donadini, associated with *Ericaceae* in the Mediterranean area, produces astipitate apothecia ≤10 mm diam. and paraphyses that are 2–3-branched at the apex (Donadini 1987).

Pseudoplectania stygia (Berk. & M.A. Curtis) Sacc. differs mainly by its small (ca 13 mm diam) apothecia and straight cylindrical paraphyses (Carbone 2013), while *P. ryvardenii* Iturr. et al. is distinguished from all other *Pseudoplectania* species by its very small (≤7 mm diam) apothecia and ornamented ascospores. Finally, *P. kumaonensis* Sanwal has distinctly agglutinated paraphyses, and *P. carranzae* (Calonge & M. Mata) M. Carbone et al. is separated from other

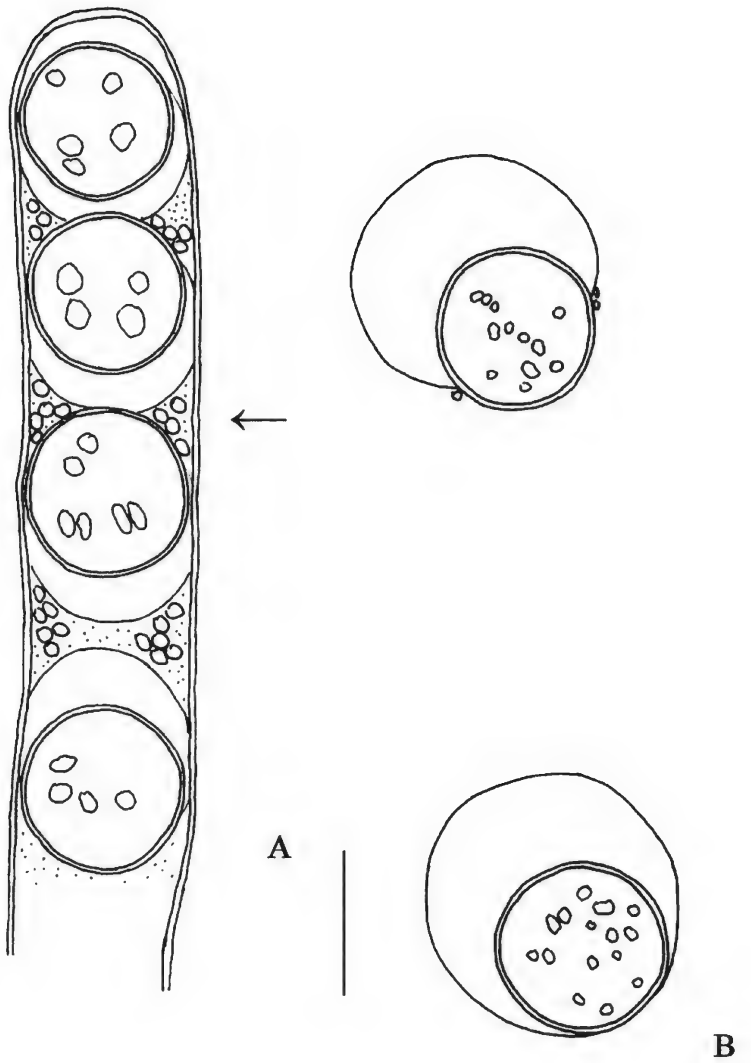

FIGURE 3. *Pseudoplectania nigrella* (SAV F-10764). **A.** Ascus with ascospores from living apothecium; cytoplasmic granules concentrated asymmetrically in a limited space by membranaceous sheath (arrowed). **B.** Ascospores with eccentrically arranged membranaceous sheath, from living apothecium. Scale bar = 10 μm.

members of the genus mainly by the presence of a tomentum or subicular hyphae and smaller asci (170–200 × 10–14 μm; Calonge & Mata 2002).

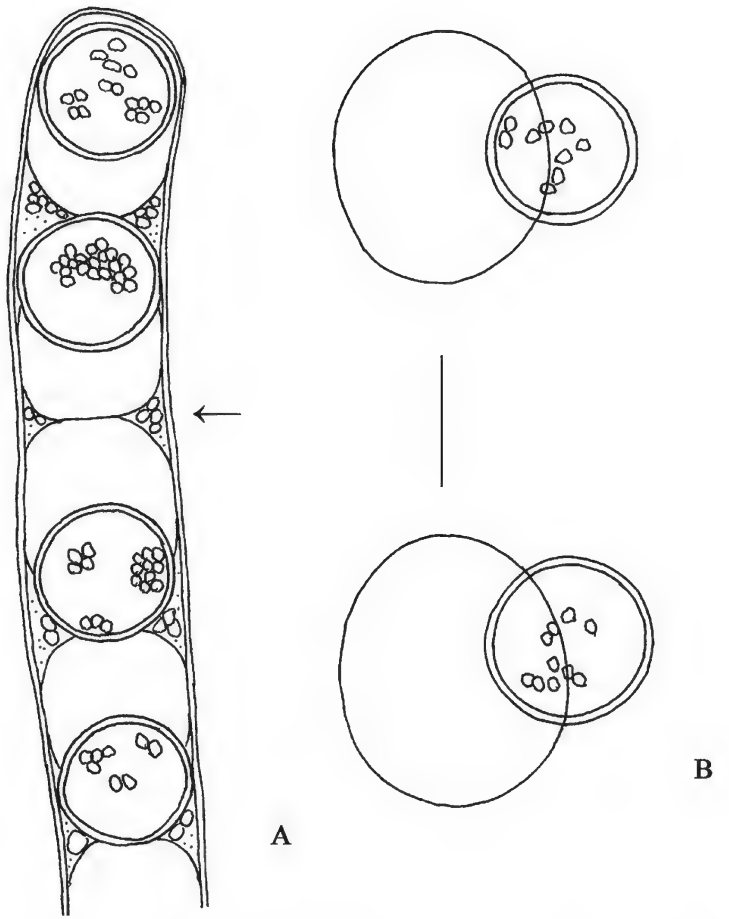

FIGURE 4. *Pseudoplectania melaena* (SAV F-10516). **A.** Ascus with ascospores from living apothecium; cytoplasmic granules concentrated asymmetrically in a limited space by membranaceous sheath (arrowed). **B.** Ascospores with laterally arranged membranaceous sheath, from living apothecium. Scale bar = 10 μm.

Based on our comparison of paraphysis tips in herbarium material of *Pseudoplectania lignicola*, *P. nigrella*, and *P. sphagnophila* with available literature data, we conclude that paraphysis shape is not helpful for distinguishing species. We observed that paraphyses are generally cylindrical, straight, and unbranched in young apothecia, whereas paraphyses in mature and senescent apothecia are branched, anastomosed, and often curved at the tip.

The nature of the membranous sheath of the spore, however, is unique for each *Pseudoplectania* taxon for which this feature has been observed. The spores of *P. lignicola* lie at the center of this sheath, and its apothecia are ≤27 mm diameter. The new species probably has a wide ecological amplitude, given its growth on *Sphagnum* sp., wood, *Leucobryum glaucum,* and bare soil. *Pseudoplectania* species usually occur on the same site for years (when the conditions allow), and we have registered and collected *P. lignicola* annually from 2009 to 2013.

In Slovakia *P. lignicola* is rare and possibly endangered (due to lodging and biotope changes) and should be included in the next edition of the Red List of Slovak fungi. The fungus has probably been overlooked and incorrectly identified in the past, but its occurrence in suitable habitats is currently not seriously threatened.

Acknowledgements

We would like to thank Peter Štefanovie for help in researching the type site of *Pseudoplectania lignicola* (Hiadeľ village), Václav Kautman for assistance in the field, Günter Bauer for providing material of *P. sphagnophila,* and Gerard Thijsse and the staff of the herbarium at Leiden for the opportunity to study Persoon's herbarium specimens. Matteo Carbone (Genova, Italy) and Ricardo Galán (Madrid, Spain), who read and commented on the manuscript, and the support of Shaun Pennycook (Auckland, New Zealand) are acknowledged. This work was supported by the VEGA [02/0088/13, 02/0150/12] for Kučera and Lizoň and by the VEGA [1/1190/12, 1/0362/13] for Glejdura and Kunca.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.11

Volume 130, pp. 11–15 *January–March 2015*

Additions to rust and chytrid pathogens of Turkey

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Abstract — *Uromyces bornmuelleri* on *Bongardia chrysogonum* and *Physoderma maculare* on *Alisma plantago-aquatica* have been recently discovered in Turkey. Morphological data obtained by light and scanning electron microscopy of identified fungi are presented.

Key words — new records, *Chytridiomycota*, *Basidiomycota*

Introduction

The *Pucciniaceae* are the largest family within the *Uredinales*, parasitising almost all major angiosperm orders, with some primarily herbaceous plant families such as *Asteraceae* and *Poaceae* being most commonly infected (van der Merwe et al. 2007). *Uromyces* (Link) Unger was raised to generic rank by Unger (1832), and is typified by *U. appendiculatus* on *Phaseolus*. Seventy-four species of *Uromyces* have been registered on 227 species of higher plants in Turkey (Bahçecioğlu & Kabaktepe 2012).

All known representatives of the genus *Physoderma* Wallr. (*Blastocladiomycetes*, *Physodermataceae*) are obligate parasites of vascular plants (Olson et al. 1980). The generalized life cycle of *Physoderma* is composed of two distinct and separate phases, and the ephemeral epibiotic sporangium with an endobiotic rhizoidal system is confined to a single host cell (Sparrow & Johns 1965). *Physoderma maculare,* the type species of the genus, on *Alisma* confirms Clinton's (1902) account of the epibiotic stage being produced from resting zoospores. The latter on mature host tissue may also give rise to the endobiotic stage, which bears resting spores. On seedlings, however, resting zoospores produce only epibiotic sporangia (Sparrow 1964).

Figs 1–7. *Uromyces bornmuelleri.* 1: Deformation of host plant. 2: Telia on leaf, in situ. 3: Teliospores. 4–6: Telia and teliospores (SEM). 7. Teliospores (SEM).

Materials & methods

Plant specimens infected with microfungi were collected from Adıyaman and Siirt provinces, Turkey and prepared according to established herbarium techniques. Host plants were identified using the Flora of Turkey and East Aegean Islands (Davis 1965–85). The fungal specimens were prepared from the host plants by obtaining thin sections. Measurements were made from tissues mounted in 5% KOH or tap water. Microscopical features were examined and microphotographs were made using a Leica

DM E light microscope. Thirty spores were measured for each sample. The microfungi were identified using relevant literature (De Toni 1888, Saccardo & Sydow 1902, Gonzalez Fragoso 1918, Kuprevich & Ulijanishchev 1975, van der Merwe et al. 2007). Species names follow Index Fungorum (2014). The examined specimens have been deposited in the mycological collection of the Department of Plant Protection, Faculty of Agriculture, Dicle University, Diyarbakır, Turkey (DUF-M).

For scanning electron microscopy (SEM), 8–10 mm squares of infected leaves were mounted on SEM stubs with double-sided adhesive tape, coated with gold using a Polaron SC 502 Sputter Coater, and examined with a Jeol JSM 6060 scanning electron microscope at 5–10 kV in the Electron Microscopy Unit, Faculty of Science, Gazi University (Turkey).

Taxonomy

Uromyces bornmuelleri Magnus, Verh. Ges. Deutsch. Naturf. 65: 151.1893. Figs 1–7

Spermogonia, aecidia and uredinia unknown. Telia amphigenous, chestnut brown, in dense groups or scattered, 0.2–3 mm diam., causing leaf deformation, at first covered by the epidermis, later becoming erumpent, pulverulent. Teliospores yellow or yellowish-brown, ovoid, globoid, sometimes oblong, 21–30 × 18–21.5 µm, rounded at the apex, rounded or sometimes attenuate at the base; wall 2–4 µm thick, pedicels hyaline, short, fragile.

Specimen examined — **TURKEY, Adıyaman Province**, Center, Kahta, Gerger, Sincik, Çelikhan, Samsat, in wheat field ecosystem, on the leaves of *Bongardia chrysogonum* (L.) Spach (*Berberidaceae*), 12.V.2009, C. Özaslan CÖ2029 (DUF-M).

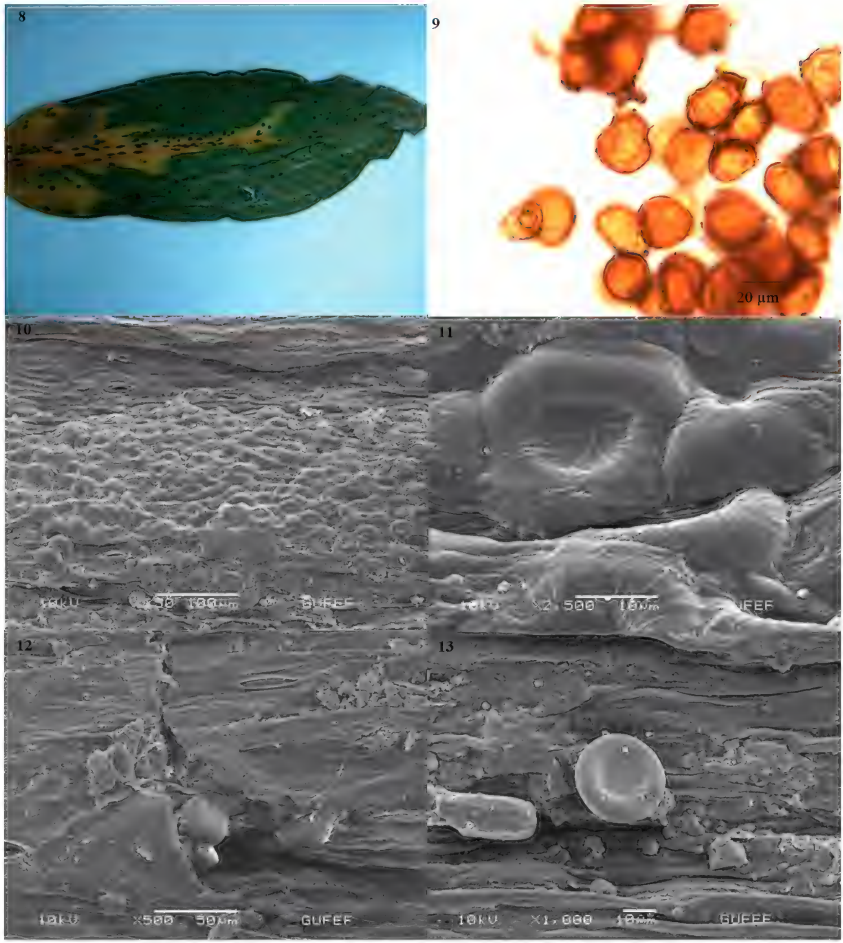
Uromyces bornmuelleri has been reported from Azerbaijan on *Leontice chrysogonum* [≡ *Bongardia chrysogonum*] (Tranzschel 1939), and from Iraq and Cyprus on *Bongardia chrysogonum* (Georghiou 1957, Mathur 1972). *Uromyces bornmuelleri* is reported for the first time from Turkey.

Physoderma maculare Wallr., Fl. Crypt. Germ. 2: 192. 1833. Figs 8–13

Resting sporangia amphigenous, mostly hypophyllous and on leaf petioles, concentrated on leaves along the nerves, scattered, covered by the unbroken epidermis, ellipsoid, ovoid, sometimes punctiformis, 1–2 mm diam., chestnut brown. Resting spores chestnut brown, globoid, ovoid to ellipsoid, content granular with more refractive globules, (23.5–)25.5–32 × 21.5–29 µm; with two distinct walls, the inner wall thin and colorless, the outer wall chestnut brown, 1–2 µm thick, smooth. Zoospores not seen.

Specimen examined — **TURKEY, Siirt Province**, Karaca Village, 38°07 09 N 42°01 59 E, in rice field ecosystem, on the leaves of *Alisma plantago-aquatica* L. (*Alismataceae*), 8.VI.2012, C. Özaslan CÖ2035 (DUF-M).

The genus *Physoderma* and *P. maculare* are reported for the first time from Turkey. This fungus has been reported from Canada (Conners 1967), Ireland



Figs 8–13. *Physoderma maculare*. 8: Resting sporangia on leaf, in situ. 9: Resting spores. 10: Resting sporangia on leaf (SEM). 11–13: Resting spores (SEM).

(Muskett & Malone 1984, as *Cladochytrium alismatis*), Poland (Czeczuga et al. 2007), Siberia (De Toni 1888, as *Uredo alismatis*), Spain (Gonzalez Fragoso 1918, as *U. alismatis*), and U.S.A. (Farr et al. 1989).

Acknowledgements

The author thanks Prof. Dr. Z.M. Azbukina (Vladivostok, Russia) and Dr. Yuri Tykhonenko (Kiev, Ukraine) for critically reading the manuscript and serving as presubmission reviewers. Especially we are grateful to Dr. Shaun Pennycook (Auckland,

New Zealand) for detailed linguistic help and nomenclatural review of the manuscript. We would like thank DÜBAP (Dicle University Research Projects Coordinator) for financial support of this Project (DÜBAP 12ZF71). We also thank Prof. Dr. Mecit Vural (Gazi University, Ankara) and Prof. Dr. A. Selçuk Ertekin (Dicle University, Diyarbakır) for his help with the identification of the hosts.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.17

Volume 130, pp. 17–25 *January–March 2015*

Dentipellicula austroafricana sp. nov. supported by morphological and phylogenetic analyses

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Abstract — *Dentipellicula austroafricana* sp. nov. is described and illustrated from South Africa based on morphological characters and rDNA sequence data. It is characterized by an annual growth habit, resupinate basidiocarps, dense soft spines, a monomitic hyphal structure with non-amyloid, non-dextrinoid and acyanophilous generative hyphae, absence of cystidia, presence of cystidioles, and tiny rough basidiospores (2.4–2.9 × 2–2.2 μm). A molecular study based on the combined ITS (internal transcribed spacer region) and nLSU (the large nuclear ribosomal RNA subunit) dataset supports the new species in *Dentipellicula*. A key to accepted species of *Dentipellicula* is provided.

Key words — *Basidiomycota, Hericiaceae*, hydnoid fungi, *Russulales,* taxonomy, wood-inhabiting fungi

Introduction

Dentipellis Donk (*Russulales, Basidiomycota*), typified by *D. fragilis* (Pers.) Donk, was introduced for species characterized by an annual growth habit, hydnoid basidiocarps, soft spines, a monomitic hyphal structure with clamp connections and cyanophilous hyphae, and amyloid rough basidiospores (Dai et al. 2009, Ginns 1986, Zhou & Dai 2013). Zhou & Dai (2013), who demonstrated that *Dentipellis* was polyphyletic, segregated *Dentipellis leptodon* (Mont.) Maas Geest. and *Dentipellis taiwaniana* Sheng H. Wu from *Dentipellis* based on ITS and nLSU rDNA sequences. They proposed *Dentipellicula* Y.C. Dai & L.W. Zhou as a new genus for these two hydnoid fungal species, which are distinguished from *Dentipellis* by their lack of cyanophilous hyphae. *Dentipellicula taiwaniana* (Sheng H. Wu) Y.C. Dai & L.W. Zhou (the generic type), and *D. leptodon* (Mont.) Y.C. Dai & L.W. Zhou currently comprise the entire genus.

[#] Jia-Jia Chen and Lu-Lu Shen contributed equally to this work and share first-author status.

During studies on the hydnoid fungi, an undescribed *Dentipellicula* species from South Africa was identified based on morphological characters and phylogenetic analysis of ITS and nLSU sequences. We provide an illustrated description and include an identification key to all three species representing *Dentipellicula*.

Materials & methods

Morphological studies

Sections were studied microscopically according to Dai et al. (2010) at magnifications ≤1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from sections stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. To present spore size variation, the 5% of measurements excluded from each end of the range are given in parentheses. Basidiospore spine lengths were not included in the measurements. Abbreviations include IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = the L/W ratio, and n = number of spores measured from given number of specimens. Special color terms follow Petersen (1996). The studied specimens are deposited in the herbaria as cited below; collection abbreviations follow Thiers (2014).

DNA extraction and sequencing

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies, Beijing) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications (Chen & Cui 2014). The DNA was amplified with the primers: ITS5 and ITS4 for ITS (White et al. 1990), and LR0R and LR7 for nLSU (Vilgalys & Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China with the same primers.

Phylogenetic analysis

New sequences, deposited in GenBank (Table 1), were aligned with additional sequences retrieved from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). *Bondarzewia podocarpi* Y.C. Dai & B.K. Cui and *B.* sp. were used as outgroup (Zhou & Dai 2013). Prior to phylogenetic analysis, ambiguous regions at the start and the end of the alignment were deleted and gaps were manually adjusted to optimize the alignment. The edited alignment was deposited at TreeBase (http://purl.org/phylo/treebase; submission ID 15859).

Table 1. Specimens used in ITS and nLSU sequence analyses.

Species	Sample no.	Locality	GenBank accession no.	
			ITS	nLSU
B. podocarpi	Dai 9261	China	KJ583207	KJ583221
Bondarzewia sp.	DAOM F-415	Canada	DQ200923	DQ234539
Dentipellicula austroafricana	**Dai 12580**	**South Africa**	**KJ855274**	**KJ855275**
D. leptodon	GB 011123	Uganda	EU118625	EU118625
D. taiwaniana	Dai 10867	China	JQ349115	JQ349101
	Cui 8346	China	JQ349114	JQ349100
Dentipellis coniferarum	Cui 10063	China	JQ349106	JQ349092
	Yuan 5623	China	JQ349107	JQ349093
D. dissita	NH 6280	Canada	AF506386	AF506386
D. fragilis	Dai 12550	China	JQ349110	JQ349096
	Dai 9009	China	JQ349108	JQ349094
D. microspora	Cui 10035	China	JQ349112	JQ349098
D. parmastoi	Cui 8513	China	JQ349113	JQ349099
Hericium abietis	NH 6990	Canada	AF506456	AF506456
H. alpestre	NH 13240	Russia	AF506457	AF506457
H. americanum	DAOM F-21467	Canada	AF506458	AF506458
H. cirrhatum	Tübingen F794	Germany	AF506385	AF506385
H. coralloides	NH 282	Sweden	AF506459	AF506459
H. erinaceus	NH 12163	Russia	AF506460	AF506460
Laxitextum bicolor	NH 5166	Sweden	AF310102	AF310102

Phylogenetic analysis followed Li & Cui (2013). Maximum parsimony (MP) analysis was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree (MPT) generated. Phylogenetic trees were visualized using Treeview (Page 1996).

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model of the combined dataset for Bayesian inference (BI). BI was calculated with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with a general time reversible (GTR)

model of DNA substitution and an invgamma distribution rate variation across sites. Four Markov chains were performed for 2 runs from random starting trees for 1 million generations of the combined ITS and nLSU dataset, and trees were sampled every 100 generations. The burn-in was set to discard the first 25% of the trees. A majority rule consensus tree of all remaining trees was calculated. Nodes that received BT support ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

To determine if the dataset was significantly conflicted, the partition homogeneity test option in PAUP 4.0b was used between the loci using 1000 replicates and the heuristic general search option. This test randomly shuffles phylogenetically informative sites between two paired loci: if the dataset is compatible, shuffling sites between the loci should not produce summed tree lengths that are significant greater than those produced by the observed data (Farris et al. 1995; Huelsenbeck et al. 1996).

Molecular phylogeny

Partition homogeneity test showed no conflicts for the two-gene combined loci (P ≥0.01). Therefore, in this study, ITS and nLSU were combined into a single analysis. The combined ITS and nLSU dataset included sequences from 20 fungal collections representing 17 species. The dataset had an aligned length of 1720 characters, of which 1246 characters are constant, 131 are variable and parsimony-uninformative, and 343 are parsimony-informative. MP analysis yielded 2 equally parsimonious trees (TL = 902, CI = 0. 708, RI = 0.786, RC = 0.557, HI = 0.292). The best model for the combined ITS and nLSU sequences dataset estimated and applied in the BI was GTR+I+G. BI resulted in a similar topology with an average standard deviation of split frequencies = 0.005368 to MP analysis, and thus only the MP tree was provided. Both BT values (≥50%) and BPPs (≥0.95) are shown at the nodes (Fig. 1).

The newly sequenced specimen from South Africa was embedded in the lineage of *Dentipellicula* (100% MP and 1.00 BPPs) and was closely related to *D. taiwaniana*.

Taxonomy

Dentipellicula austroafricana Jia J. Chen, L.L. Shen & Y.C. Dai, **sp. nov.** Fig. 2

MycoBank MB 811108

Differs from other *Dentipellicula* species by its exclusively resupinate basidiocarps, dense spines, absence of gloeoplerous hyphae and cystidia, presence of cystidioles, and tiny rough basidiospores.

Type — South Africa, KwaZulu-Natal Province, Durban, on charred wood of *Ficus*, 1.X.2011, Dai 12580 (**holotype**, BJFC; **isotype**, IFP; GenBank KJ855274, KJ855275).

Etymology — *austroafricana* (Lat.): referring to South Africa.

Fruitbody — Basidiocarps annual, resupinate, inseparable, hard corky upon drying, up to 6 cm long, 2 cm wide, 3 mm thick at center, without odor or taste.

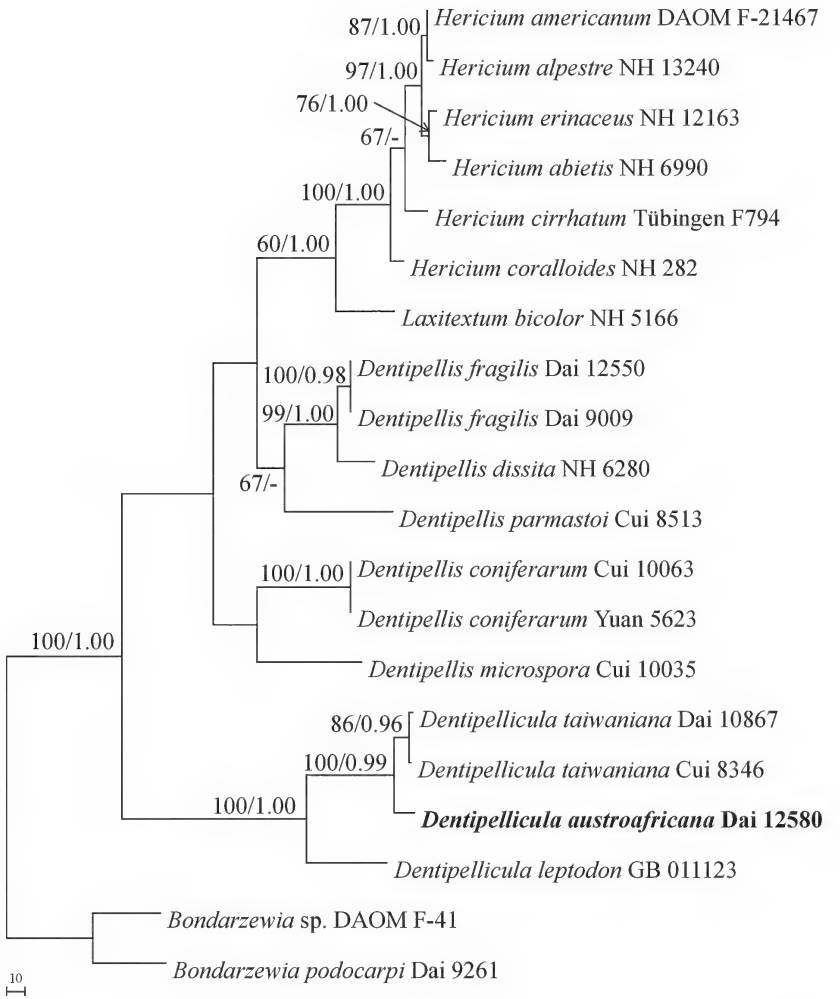

FIG. 1. Strict consensus tree illustrating the phylogenetic position of *Dentipellicula austroafricana*, generated by maximum parsimony method based on ITS+nLSU sequence data. Branches are labeled with parsimony bootstrap values ≥50% and Bayesian posterior probabilities ≥0.95.

Fresh spines soft, white to cream, when dry fragile, cream to buff, up to 2 mm long, 8–10 per mm across base. Margin cottony, buff to clay-buff, ≤1 mm wide. Subiculum very thin, soft corky, buff to reddish brown, ≤1 mm thick.

HYPHAL STRUCTURE — Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

Subiculum — Generative hyphae colorless, thick-walled, frequently branched, interwoven, 4–6 µm in diam. Gloeoplerous hyphae absent.

Hymenophoral trama — Generative hyphae colorless, slightly thick-walled to thick-walled, moderately branched, more or less subparallel along the spines, 2–5.5 µm in diam. Gloeoplerous hyphae absent.

Hymenium — Cystidia absent; fusoid cystidioles present, hyaline, thin-walled, 16.5–18 × 1–4 µm; basidia clavate with four sterigmata and a basal clamp connection, 18–23 × 3.5–5 µm; basidioles similar to basidia in shape, but smaller than basidia. Basidiospores ellipsoid to broadly ellipsoid, thick-walled, colorless, minutely rough, strongly amyloid, CB+, 2.4–2.9(–3) × (1.8–)2–2.2 (–2.4) µm, L = 2.83 µm, W = 2.12 µm, Q = 1.29–1.39 (n = 60/1).

Other species examined — ***Dentipellicula leptodon*: CANADA. Ontario province, Algonquin Nat. Res.**, on *Betula*, 22.X.1966 (DAOM F-158439).

***Dentipellicula taiwaniana*: CHINA. Hainan province, Ledong County,** Jianfengling Nat. Res., on fallen angiosperm trunk, 12.V.2009, Dai 10872 (BJFC 5114).

Key to accepted species of *Dentipellicula*

1. Basidiospores >3.5 µm long. . . . . *D. leptodon*
1. Basidiospores <3.5 µm long. 2
2. Spines 5–7 per mm; cystidia present . . . . . *D. austroafricana*
2. Spines 8–10 per mm; cystidia absent . . . . . *D. taiwaniana*

Discussion

Phylogenetically, *Dentipellicula austroafricana* is recognized in the *Dentipellicula* clade and distant from *Dentipellis, Hericium* Pers., and *Laxitextum* Lentz based on the combined ITS and nLSU sequences (Fig. 1). Morphologically, *D. austroafricana* is characterized by an annual growth habit, resupinate basidiocarps, soft and dense spines, a monomitic hyphal structure with non-dextrinoid generative hyphae, presence of cystidioles, and tiny rough basidiospores. Both morphology and rDNA sequence data confirmed that *D. austroafricana* is a new species in *Dentipellicula*.

According to the ITS and nLSU-based phylogeny (Fig. 1), *Dentipellicula austroafricana* is closely related to *D. taiwaniana,* which also has annual basidiocarps and soft spines but which is distinguished by its effused-reflexed basidiocarps, more scattered spines (5–7 per mm), narrower (2–3.5 µm) generative hyphae in the subiculum, presence of gloeocystidia, and longer basidiospores (2.8–3.4 × 2.1–2.4 µm; Dai et al. 2009, Wu 2007).

Dentipellicula leptodon also has annual basidiocarps and soft spines but differs from *D. austroafricana* in its narrower (2–4 µm) generative hyphae in the subiculum, presence of gloeopleurous hyphae in the subiculum and gloeocystidia, and larger basidiospores (3.6–4.2 × 2.8–3.3 µm; Dai et al. 2009,

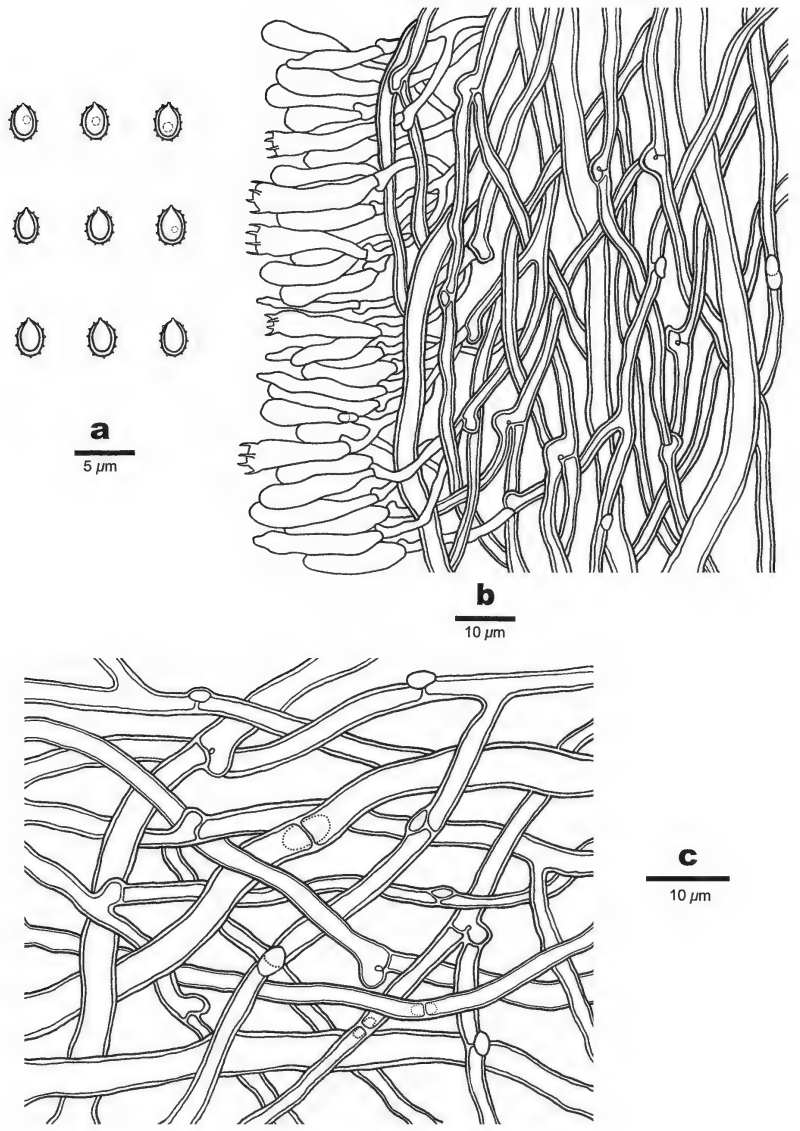

Fig. 2. *Dentipellicula austroafricana* (holotype).
a. Basidiospores. b. Vertical section through a spine. c. Hyphae from subiculum.

Ginns 1986). Moreover, the two species are separated in the ITS and nLSU-based phylogenetic analysis (Fig. 1).

Our phylogenetic tree was overall consistent with that presented by Zhou & Dai (2013): for now, *Dentipellis* remains polyphyletic based on ITS and nLSU rDNA sequences, while the new segregate genus *Dentipellicula* is monophyletic (Fig. 1). A fully resolved phylogeny for *Dentipellis* and its related genera requires evolutionary information from wider taxa samplings and more conserved gene markers.

Acknowledgements

We express our gratitude to Drs. Li-Wei Zhou (Institute of Applied Ecology, Chinese Academy of Sciences, China) and Michal Tomšovský (Mendel University in Brno, Czech Republic) who reviewed the manuscript. The research was financed by the Fundamental Research Funds for the Central Universities (No. BLYJ201403).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.27

Volume 130, pp. 27–31 *January–March 2015*

A new species of *Terriera* (*Rhytismatales, Ascomycota*) on *Photinia villosa*

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Abstract — A new *Terriera* species, *T. aequabilis* on fallen leaves of *Photinia villosa* from the Mount Sanqingshan National Park, Jiangxi Province, China, is described, illustrated, and discussed. This taxon is distinguished from *T. cladophila* by smaller ascomata that are not associated with conidiomata, black thin zone lines, a well-developed excipulum, and unbranched paraphyses. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — taxonomy, morphology, *Rhytismataceae*, *Rosaceae*

Introduction

Eriksson (1970) established the genus *Terriera* based on *T. cladophila* (Lév.) B. Erikss. (≡ *Hysterium cladophilum* Lév.), which produces a different paraphysial type and opening mechanism from genera *Lophodermium* Chevall. and *Sporomega* Corda. Minter (1996) provided a detailed description for *T. cladophila*. Johnston (2001), who systematically studied monocotyledon-inhabiting *Lophodermium*, divided that heterogeneous genus into five groups, one of which was transferred to *Terriera*. IndexFungorum (2014) lists 25 specific and two varietal epithets in *Terriera.*

Here, *Terriera aequabilis* on fallen leaves of oriental photinia from Jiangxi Province, China, is described as a new species.

Materials & methods

Mature ascomata were selected from the collected specimens. Under the dissecting microscope with a 10–50× magnification, the macroscopic appearance of ascomata and zone lines was observed. After rehydration of reference materials in water for 15 min., vertical transverse 10–15μm thick sections were sliced from ascomata using a freezing microtome and mounted in 0.5% (w/v) cotton blue in water for observing ascomatal

outlines. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) lactophenol-cotton blue. The color of internal structures and ascospore contents were observed in water. Squash mounts were prepared in 5% KOH solution for measurements of asci, ascospores, and paraphyses. Figures of the external shapes and internal structures of the fruitbodies were drawn using a microscopic painting tube. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Taxonomy

Terriera aequabilis Qing Li & Y.R. Lin, **sp. nov.** FIGS 1–5

MycoBank MB 808818

Differs from *Terriera cladophila* by smaller ascomata not associated with conidiomata, frequent black and thin zone lines, a much thinner covering stroma, unbranched paraphyses swollen conspicuously at the apex, and ascospores with a visible mucous sheath.

TYPE: China, Jiangxi, Mount Sanqingshan National Park, Bingyudong, alt. 1550 m, on fallen leaves of *Photinia villosa* (Thunb.) DC. (*Rosaceae*), 21 August 2012, Y.R. Lin, S.J. Wang & L. Zhang 2657 (**Holotype**, AAUF 68765).

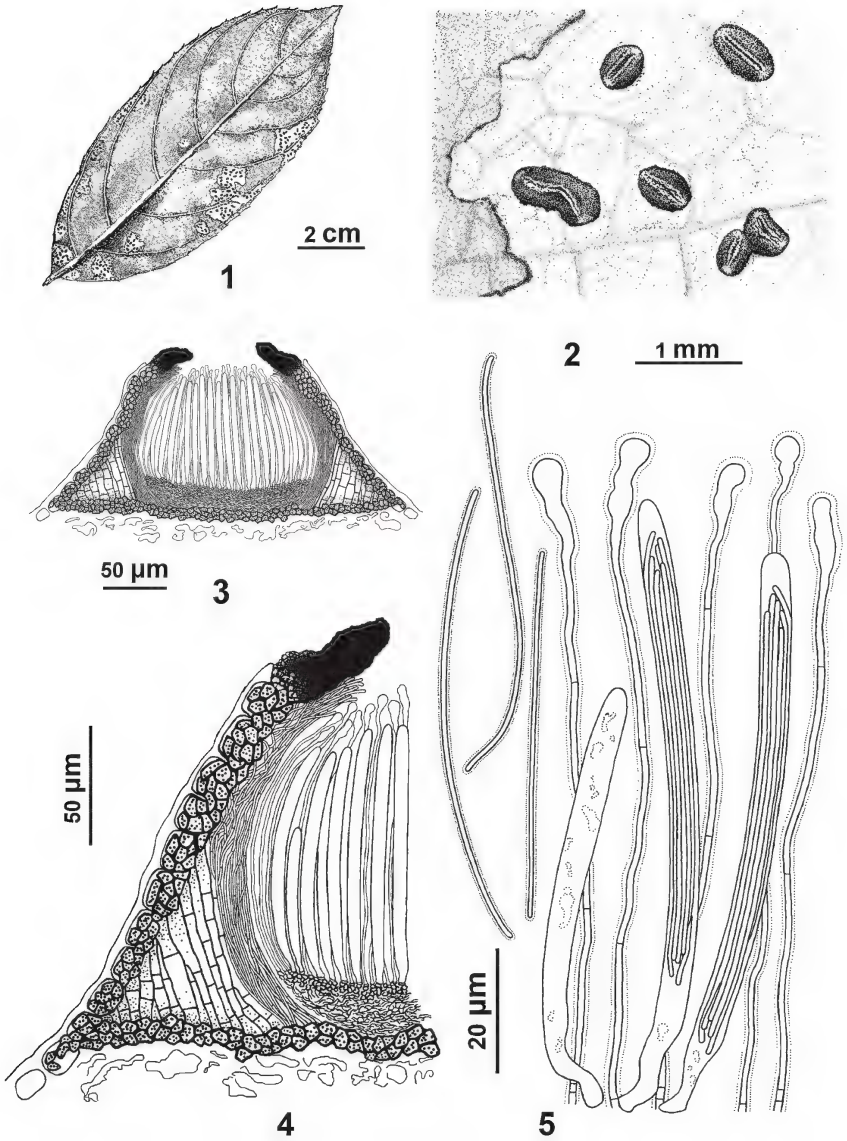
ETYMOLOGY: *aequabilis* (Latin = uniform), referring to the single ascospore, which has an equal diameter throughout its length.

COLONIES on both sides of leaves, forming distinct subcircular or irregular, yellow-brown bleached areas each with an obvious edge, 6–10 mm diam., which tend to coalesce into larger irregular shapes.

ZONE LINES usually frequent, dark brown or black, thin, entirely or partly surrounding the paler areas.

CONIDIOMATA not observed.

ASCOMATA developing on both sides of leaves, predominantly on the lower side, scattered over the pale areas, with two ascomata sometimes coalescent. In surface view, ascomata 320–720 × 250–430 µm, elliptical to subcircular, straight or slightly curved to one side, ends rounded. Whole surface of ascoma black, except sometimes for a paler region at each end, with a clearly defined edge, slightly shiny, moderately raising above the substrate surface but somewhat sunken near the split, opening by a single longitudinal split extending most of the length of the ascoma. Immature ascomata appearing as a single dark brown protrusion, more or less rotund in outline, not forming a pale zone along the future line of opening. In median transverse vertical section, ascomata subepidermal with epidermal cells becoming filled with fungal tissue as ascoma develops, 165–190 µm deep. COVERING STROMA 15–20 µm thick near the opening, becoming thinner towards the edge and connecting to the basal stroma, composed mainly of textura angularis with blackish-brown to dark brown, thick-walled cells 3–5.5 µm diam. Lip cells absent. Along the edge of the ascoma opening, there is a short extension, 10–18µm thick, adjacent



Figs 1–5. *Terriera aequabilis* (holotype, AAUF 68765) on *Photinia villosa* 1. Habit on a leaf. 2. Detail of ascomata and a zone line. 3. Ascoma in transverse vertical section. 4. Portion of ascoma in transverse vertical section. 5. Paraphyses, asci, and ascospores.

to the top of the covering stroma, which is comprised of strongly carbonized tissue with no obvious cellular structure. Excipulum well-developed, 10–15 µm thick, arising from the inner layer of the basal stroma, septate, comprised of colorless textura porrecta-intricata with hyphae ca 0.8 µm diam., sometimes a reticular structure formed by the interlaced hyphae at the upper part. Basal stroma 6–10 µm thick, dark brown, consisting of 2(–3) layers of 3–5 µm diam., angular, thick-walled cells. Colorless to grayish-brown textura prismatica with cell 3–8.5 µm diam., 20–45 µm thick, existing in the triangular area, visible in sections between the covering and basal stroma at each edge of the ascoma. Subhymenium 15–22 µm thick, rather flat, consisting of hyaline textura-angularis and textura intricata. Paraphyses 1–1.3 µm wide, filiform, sparsely septate, not branched, gradually or suddenly swollen to 2.8–5 µm at the apex, extending ca 10 µm beyond asci, immersed in a ca 0.5 µm thick gelatinous matrix, hyphal bridges no observed at the base. Asci maturing sequentially, cylindric-clavate, 75–105 × 4.5–5.5 µm, short-stalked, thin-walled, apex round or slightly obtuse, J-, 8-spored. Ascospores arranged fasciculately, with a uniform diameter throughout the single ascospore, 55–78 × 0.8–1 µm, filiform, hyaline, aseptate, ends rounded, covered by a 0.3–0.5 µm thick gelatinous sheath.

Additional specimens examined — On dead leaves of *Photinia villosa*: **China, Jiangxi:** Mount Sanqingshan National Park, Huaiyushan, alt. ca 600 m, 18 August 2012, S.J. Wang & Y.R. Lin 2632 (AAUF 68740); Sanmucun, alt. ca 850 m, 20 August 2012, F. Zhou & L. Zhang 2645 (AAUF 68753); Yulinfeng, alt. ca 810 m, 21 August 2012, Y.R. Lin, S.J. Wang & L. Zhang 2704 (AAUF 68812).

Comments — *Terriera aequabilis* is somewhat similar to the type species *T. cladophila* in the shape of ascomata, asci, and ascospores. However, *T. cladophila* occurs on dead or living twigs and has larger (350–900 × 300–600 µm) ascomata that are associated with conidiomata, a much thicker (≤40 µm) covering stroma composed of textura globulosa or angularis, paraphyses with frequently and irregularly branched and irregularly twisted swollen upper parts, sometimes with hyphal bridges at the base between adjacent paraphyses, ascospores without mucous sheath, and brown diffuse zone lines occasionally present (Minter 1996).

Terriera minor (Tehon) P.R. Johnst. [≡ *Lophodermium minus* (Tehon) P.R. Johnst.], the most widely distributed species, differs from the new species in oblong to oblong-elliptic, paraphyses branching 2–3 times in upper 30–40µm, extend 20–30 µm beyond asci and sometimes irregularly swollen at the apices, longer and wider (100–130 × 6–7 µm) asci that are tapering abruptly to small, rounded apex, wider (1.5–2 µm) ascospores slightly tapering towards both ends and 0–1-septate, and a poorly developed subhymenium (Johnston 1989). In addition, Tehon's (1918) observation that a thin, hyaline epithecium was formed by apically coiled paraphyses differs from Johnston's description.

The similar *Terriera simplex* Y.L. Lin et al. is distinguished from *T. aequabilis* by larger (650–1000 × 350–480 μm), sometimes triangular ascomata with conidiomata, a very poorly developed excipulum, wider (1.2–1.5 μm) paraphyses occasionally swollen to 2.5–3 μm near the apex, synchronously maturing asci with truncate to obtuse apices, and ascospores tapering to the rounded base (Gao et al. 2012).

Acknowledgements

The authors are grateful for the pre-submission comments and suggestions provided by Dr Z. Wang (Yale University, USA) and Dr M. Ye (Hefei University of Technology, China) and to F. Zhou and L. Zhang for the field investigations. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31170019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.33

Volume 130, pp. 33–40 *January–March 2015*

New records of one *Amygdalaria* and three *Porpidia* taxa (*Lecideaceae*) from China

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Abstract —Four lichen taxa of *Lecideaceae*, *Amygdalaria consentiens* var. *consentiens*, *Porpidia carlottiana*, *P. lowiana*, and *P. tuberculosa*, are reported for the first time from China.

Key words —Asia, *Lecideales*, lichens

Introduction

The family *Lecideaceae* Chevall. contains about 23 genera and 547 species, of which the largest genus is *Lecidea* Ach., containing about 427 species (Kirk et al. 2008, Fryday & Hertel 2014). In China, twenty-three species of the other genera in *Lecideaceae* have been reported, including two each of *Amygdalaria* Norman, *Bellemerea* Hafellner & Cl. Roux, and *Immersaria* Rambold & Pietschm.; one each of *Lecidoma* Gotth. Schneid. & Hertel, *Paraporpidia* Rambold & Pietschm., and *Stenhammarella* Hertel; and 16 species of *Porpidia* Körb. (Hertel 1977, Wei 1991; Aptroot & Seaward 1999; Aptroot 2002; Aptroot & Sparrius 2003; Obermayer 2004; Guo 2005; Zhang et al. 2010, 2012; Wang et al. 2012; Ismayi & Abbas 2013; Hu et al. 2014).

Amygdalaria and *Porpidia* are obviously very closely related. Both have large halonate ascospores, a high hymenium, *Porpidia*-type asci, and a dark pigmented hypothecium and are (with a few exceptions) restricted to lime-free, silicate rocks. However *Amygdalaria* can be best distinguished from *Porpidia* by the presence of cephalodia, the higher hymenium (over 130 µm), the larger ascospores (generally 20–35 × 10–16 µm) with conspicuous, rather compact epispores, and a tendency toward a brownish or yellowish pink thallus (Inoue 1984, Brodo & Hertel 1987, Gowan 1989, Smith et al. 2009).

During our research on the lecideoid taxa of *Lecideaceae* in China, we have identified four taxa new to the country: *Amygdalaria consentiens* var. *consentiens*, *Porpidia carlottiana*, *P. lowiana*, and *P. tuberculosa*.

Materials & methods

The studied specimens are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU); Herbarium Mycologicum Academiae Sinicae – Lichenes, Beijing, China (HMAS-L); and the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN). The morphological and anatomical characters were examined under a stereomicroscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41). The thalli and medullae were tested for identification with K (10% aqueous KOH solution), C (saturated aqueous NaClO solution), I (a 10% aqueous KI solution), P (a saturated *p*-phenylenediamine solution in 95% ethyl alcohol). The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent system C (Orange et al. 2010). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Amygdalaria consentiens (Nyl.) Hertel, Brodo & Mas. Inoue, J. Hattori Bot. Lab. 56: 322 (1984) **var. *consentiens*** Fig. 1

Morphology — Thallus continuous, cracked-areolate, usually thick, light orange to brownish gray, often becoming oxidated orange; medulla I–; prothallus black, thin and conspicuous; cephalodia mostly flush with thallus surface, almost the same colour as the thallus, but occasionally pinker or gray, containing *Stigonema;* soredia absent. Apothecia entirely sunken in thallus, (0.35–)0.45–0.9 mm diam., usually one or two per areole; disc dark brown to black, margin slightly to moderately rusty orange pruinose. Exciple black-brown, 50–70 µm wide, without crystals; epihymenium brownish, with orange crystals; hymenium hyaline, 150–200 µm tall; subhymenium colorless, 45–60 µm tall; hypothecium blackish brown, usually thin, bowl-shaped; paraphyses strongly anastomosed and branched, almost moniliform at the apical part, with ±swollen apices. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 28–32(–35) × 14–17(–19) µm, halonate. Pycnidia immersed, conidia bacilliform, 6–9 × c. 1 µm.

Chemistry — Thallus and medulla K–, C–, KC–, P–. No lichen substances were detected by TLC.

Distribution — *Amygdalaria consentiens* has been reported from Asia, Europe, and North America (Inoue 1984, Brodo & Hertel 1987, Smith et al. 2009). New to China.

Specimen examined: **CHINA. Yunnan**, Luquan, Mt. Jiaozixueshan, alt. 3800 m, on rock, 26 Oct. 2008, Z.J. Ren 20108488 (SDNU).

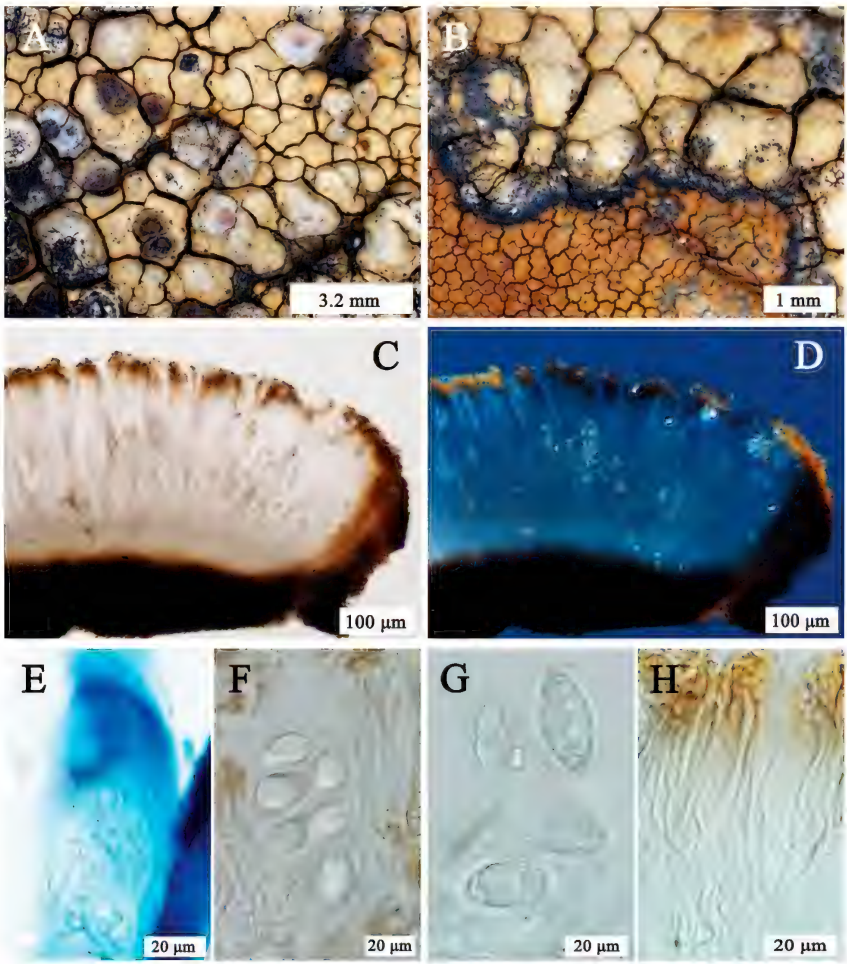

Fig. 1 *Amygdalaria consentiens* var. *consentiens* (Ren 20108488, SDNU). A: thallus; B: prothallus; C: apothecium section; D: epihymenium with crystals; E: amyloid reaction of ascus; F: ascus and ascospores; G: ascospores; H: paraphyses.

Comments —*Amygdalaria consentiens* var. *consentiens* is morphologically similar to *A. consentiens* var. *japonica* and *A. continua*, but *A. consentiens* var. *japonica* contains stictic and constictic acids, while *A. continua* has a smooth to finely rimose thallus which is rarely cracked-areolate.

Porpidia carlottiana Gowan, Bryologist 92: 39 (1989) Fig. 2

Morphology — Thallus crustose, finely cracked to subrimose, 0.15–0.25 mm thick, whitish gray; medulla I–; prothallus continuous between thallus

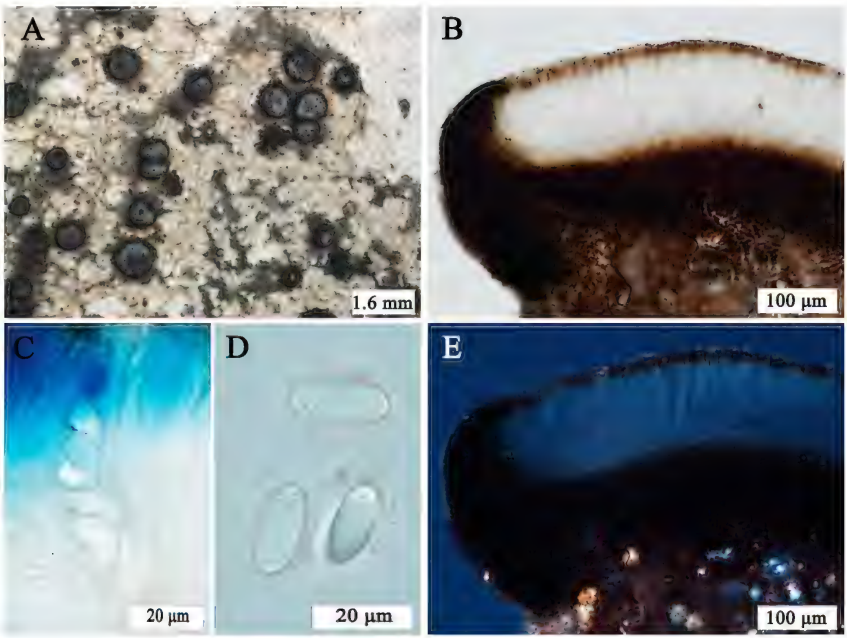

FIG. 2 *Porpidia carlottiana* (Wang 20127123, SDNU). A: thallus; B: apothecium section; C: amyloid reaction of ascus; D: ascospores; E: exciple and epihymenium without crystals.

patches, black, thin; soredia absent. APOTHECIA scattered or contiguous, sunken in thallus when young, becoming sessile on a broad base when mature, 0.5–1.1(–1.5) mm diam.; disc black, plane to weakly convex, usually with heavy whitish pruina; margin bare, distinct, regular to flexuose. EXCIPLE dark brown to black at margin, brown within, 90–105 µm wide, without crystals; epihymenium yellowish brown or olive brown, without crystals; hymenium hyaline, 90–105(–130) µm tall; subhymenium 20–25 µm, hypothecium blackish brown; paraphyses strongly anastomosed and apically branched. ASCI clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 17–20 × 7–10 µm, halonate. PYCNIDIA not observed.

CHEMISTRY — Thallus and medulla K–, C–, KC–, P–. 2'-O-methyl-superphyllinic acid was detected by TLC.

DISTRIBUTION — *Porpidia carlottiana* has been reported from North America (Gowan 1989). New to China.

SPECIMEN EXAMINED: **CHINA. YUNNAN**, Lijiang, Mt. Laojunshan, alt. 3800 m, on rock, 5 Nov. 2009, H.Y. Wang 20127123 (SDNU).

COMMENTS — *Porpidia carlottiana* is closely related to *P. rugosa*, which also produces pruinose apothecia and 2'-O-methylsuperphyllinic acid. However, *P. rugosa* always has soredia, and the apothecia rarely seen.

Porpidia lowiana Gowan, Bryologist 92: 49 (1989) FIG. 3

MORPHOLOGY — THALLUS crustose, continuous, finely cracked to rimose-areolate, 0.2–0.5 mm thick, yellowish gray to whitish, sometimes dark gray or greenish gray; medulla I–; prothallus absent; soredia absent. APOTHECIA scattered to contiguous, soon becoming broadly sessile, 0.5–1.3 mm diam.; disc black, non-pruinose to moderately pruinose; margin bare, distinct, even to weakly crenulate, regular to flexuose. EXCIPLE mostly black and carbonaceous at exciple margin, hyaline within, 55–90 µm wide, with crystals that are soluble in K and C; epihymenium olive brown, without crystals; hymenium hyaline, 70–110 µm tall; subhymenium 18–26 µm, hypothecium blackish brown; paraphyses strongly anastomosed and apically branched. ASCI clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid or tear-drop-shape, 14–16(–25) × (4.5–)6–8 µm, halonate. PYCNIDIA not observed.

CHEMISTRY — Thallus and medulla K–, C–, KC–, P–. Confluentic, 2'-O-methylmicrophyllinic, and 2'-O-methylperlatolic acids were detected by TLC.

DISTRIBUTION — *Porpidia lowiana* has been reported from Europe and North America (Gowan 1989, Smith et al. 2009). New to China.

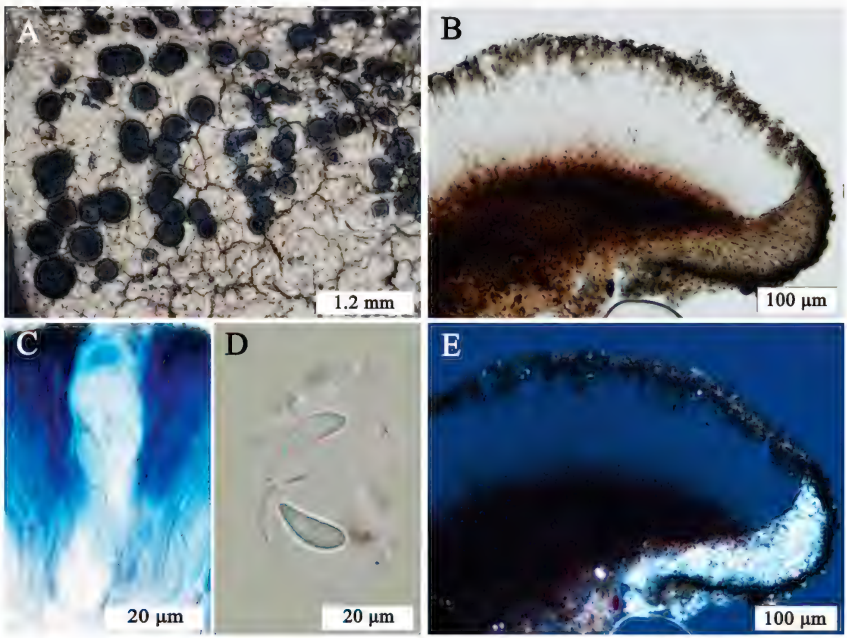

FIG. 3 *Porpidia lowiana* (Ren 20102097, SDNU). A: thallus; B: apothecium section; C: amyloid reaction of ascus; D: ascospores; E: exciple with crystals.

Specimens examined: **CHINA. Helongjiang**, Tahe, Mt. Mengkeshan, alt. 555 m, on rock, 14 Aug. 2009, Q. Ren 20102097 (SDNU). **Inner Mongolia**, Mangui, alt. 900 m, on rock, 13 Sep. 1977, J.C. Wei, 074581 (HMAS-L); **Sichuan**, Dukou, Mt. Dabaoding, alt. 1950 m, on rock, 21 Jun. 1983, L.S. Wang 83-184 (KUN); **Yunnan**, Xinping, Mt. Mopan, alt. 2000 m, on rock, 20 Dec. 2008, L.S. Wang 08-30003 (KUN); Chuxiong, Mt. Zixi, alt. 2060 m, on rock, 31 Aug. 2005, L.S. Wang 05-25263 (KUN); Lincang county, Matai village, alt. 1650 m, on rock, 20 Oct. 2003, L.S. Wang 03-22921, 03-22922 (KUN); Yimen, alt. 1600 m, on rock, 10 Dec. 1990, L.S. Wang 11707 (KUN).

Comments — *Porpidia lowiana* is closely related to *P. cinereoatra*, from which it differs mainly in having a thinner, yellower thallus, thicker proper margin (c. 0.05 mm in *P. cinereoatra*), and sessile apothecia (mainly sunken in *P. cinereoatra*).

Porpidia tuberculosa (Sm.) Hertel & Knoph, Beih. Nova Hedwigia 79: 438 (1984)

Fig. 4

Morphology — Thallus crustose, continuous, subrimose to distinctly rimose-areolate, 0.2–0.3(–0.5) mm thick, medium gray or sometimes rusty orange; medulla I+ violet; prothallus usually present when abutting another lichen, black; soralia present, 0.3–0.7 mm diam., round to irregular, scattered to crowded, shallowly erose or abraded, usually with slightly raised rim; soredia farinose to granular, white, pale gray or flecked blue-gray or blackish, I+ violet. Apothecia occasional, scattered, soon becoming sessile, 0.9–1.5 (–1.7) mm diam.; disc black, plane to subconvex, weakly to moderately pruinose; margin bare, distinct, regular to flexuose. Exciple greenish to brownish black at exciple margin, dark brown within, 90–140 µm wide, without crystals; epihymenium olive brown, without crystals; hymenium hyaline, 90–105 µm tall; subhymenium 35–50 µm, hypothecium blackish brown; paraphyses strongly anastomosed and apically branched. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 17.5–22 × 7–8 µm, halonate. Pycnidia immersed, conidia bacilliform, 7.5–10 × c. 1 µm.

Chemistry — Thallus and medulla K–, C–, KC–, P–. Confluentic, 2'-O-methylmicrophyllinic, and 2'-O-methylperlatolic acids were detected by TLC.

Distribution — *Porpidia tuberculosa* has been reported from Asia, Macaronesia, Europe, and North & South America (Hertel 1984, Smith et al. 2009). New to China.

Specimens examined: **CHINA. Hubei**, Shennongjia, Mt. Dashennongjia, alt. 3000 m, on rock, 27 Jun. 1984, J.C. Wei, 067994, 068005, 074292 (HMAS-L). **Yunnan**, Luquan, Mt. Jiaozixueshan, alt. 3800 m, on rock, 27 Oct. 2008, H.Y. Wang 20081180, 20082302 (SDNU).

Comments — *Porpidia tuberculosa, P. rugosa* and *P. soredizodes* all have a thallus with soralia. However, *P. rugosa* has a more verrucose thallus,

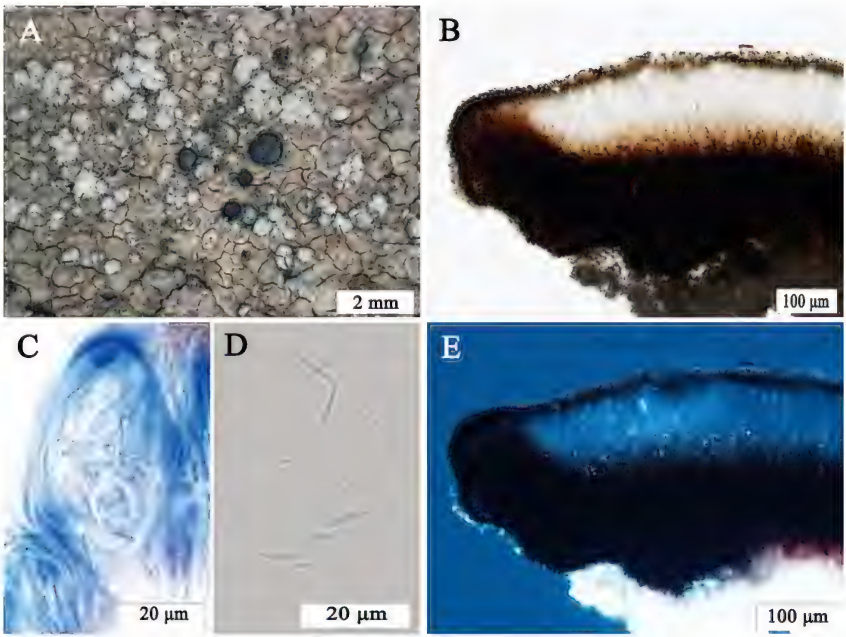

FIG. 4 *Porpidia tuberculosa* (Wei 067994, HMAS-L). A: thallus; B: apothecium section; C: amyloid reaction of ascus; D: conidia; E: exciple and epihymenium without crystals.

I– medulla, and contains 2'-O-methylsuperphyllinic and glaucophaeic acids. *Porpidia soredizodes* has an I– medulla as well as an overall smaller thinner (≤0.2 mm) darker thallus that contains stictic acid.

Acknowledgements

The authors thank Dr. A. Aptroot (ABL Herbarium, Soest, the Netherlands) and Dr. Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for presubmission reviews. This study was supported by Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province, the National Natural Science Foundation of China (31170187), Foundation of Key Laboratory, CAS (KLBB–201306), and the Scientific Research Foundation of Graduate School of Shandong Normal University (BCX1406).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.41

Volume 130, pp. 41–46 *January–March 2015*

Anungitea guangxiensis and *Ellisembia longchiensis,* two new species from southern China

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Abstract — Two new hyphomycete species, *Anungitea guangxiensis* and *Ellisembia longchiensis,* are described and illustrated from specimens collected on dead branches in southern China. *Anungitea guangxiensis* is characterized by conidiogenous cells that are polyblastic, integrated, terminal, smooth, and denticulate (with cylindrical denticles) and conidia produced in acropetal chains. *Ellisembia longchiensis* is characterized by distinct, single, unbranched, erect conidiophores, monoblastic conidiogenous cells, and distoseptate conidia that have a small, globose, mucilaginous apical sheath.

Key words — conidial fungi, taxonomy

Introduction

Anungitea was established by Sutton (1973) as a monotypic genus with *A. fragilis* B. Sutton as the type species. The genus is characterized by conidia borne on conspicuous denticulate loci of the terminal or intercalary conidiogenous cells and which form acropetal, unbranched chains. A further 19 names have been proposed in *Anungitea* (Castañeda 1986; Castañeda & Kendrick 1990, 1991; Castañeda et al. 1997; Crous et al. 1995, 2014; Kirk 1983; Matsushima 1975; Rambelli et al. 2008, 2009, 2010; Sutton & Hodges 1978), but subsequently *A. globosa* B. Sutton & Hodges was synonymised with *A. fragilis* (Kirk 1982; Matsushima 1987; Castañeda Ruíz et al. 1996), and *A. triseptata* R.F. Castañeda & W.B. Kendr. was transferred to *Anungitopsis* (Castañeda & Kendrick 1991).

The genus *Ellisembia* was introduced by Subramanian (1992) to accommodate *Sporidesmium*-like species with determinate or irregularly percurrently extending conidiogenous cells that produce distoseptate conidia. Wu & Zhuang (2005) merged *Imicles* Shoemaker & Hambl. (Shoemaker & Hambleton 2001) into *Ellisembia* and expanded the generic concept to include species that produce typically lageniform, ovoid, or doliiform percurrently extending conidiogenous cells.

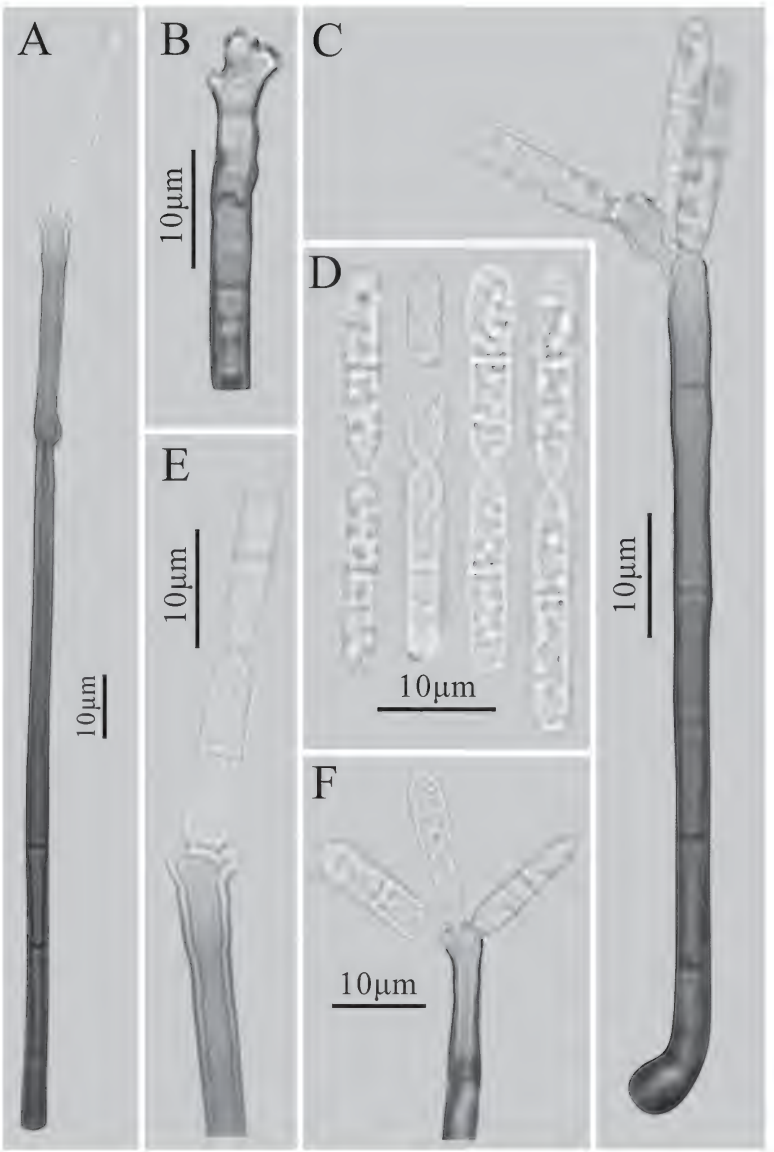

Fig. 1. *Anungitea guangxiensis* (holotype, HSAUP H6351).
A,C. Conidiophores with conidia. B. Apex of conidiophore.
D. Conidia. E,F. Apex of conidiophores with conidia.

Many taxa of hyphomycetes have been already described from China (Wu & Zhuang 2005, Zhang et al. 2009a,b, 2011, 2012, Ma et al. 2010, 2012a,b,c, Ren

et al. 2012). In our studies on hyphomycetes from deciduous stems and rotten wood in southern China, we collected two hyphomycetes that possess the morphological characteristics of *Anungitea* and *Ellisembia*. Both are proposed herein as new species.

Anungitea guangxiensis J.W. Xia & X.G. Zhang, **sp. nov.** FIG. 1

MycoBank MB 811157

Differs from *Anungitea heterospora* by its much larger conidia; and from *A. longicatenata* by its longer, cylindrical conidia.

TYPE: China, Guangxi Province: Mount Dayao, on dead stems of unidentified broad-leaved tree, 26 Oct 2012, J.W. Xia (**Holotype**, HSAUP H6351; **isotype**, HMAS 243458).

ETYMOLOGY: in reference to the province where the type was found.

Colonies on natural substrate effuse, pale brown to brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of septate, pale brown, smooth, 1–2 µm wide hyphae. Conidiophores distinct, single, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, thick-walled, pale brown to brown, 6–11-septate, 70–160 × 2.5–4.5 µm. Conidiogenous cells polyblastic, integrated, terminal, sympodial, hyaline to pale brown, 10–18 × 2.5–4.5 µm, with 1–6 short denticles. Conidia acrogenous, cylindrical, in unbranched acropetal chains, dry, subhyaline to hyaline, 1-septate, truncate at the base and rounded at the apex, 16–22 µm long, 3–4 µm wide.

COMMENTS – Among the known species, *Anungitea guangxiensis* bears some resemblance to *A. heterospora* P.M. Kirk and *A. longicatenata* Matsush. in conidial shape. However, the primary conidia of *A. heterospora* are smaller (8–9.5 × 3–4 µm, 1-septate) than those of *A. guangxiensis* and occasionally slightly constricted at the septum (Kirk 1983). The conidia of *A. longicatenata* differ by being shorter (11–18 × 2.5–4 µm, 0–2-septate), narrowly obovoid to cylindrical, and tapering at both ends (Matsushima 1975).

Ellisembia longchiensis J.W. Xia & X.G. Zhang, **sp. nov.** FIG. 2

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Differs from *Ellisembia mucicola* by its longer, narrower, obclavate conidia with more numerous distosepta; and from *E. suttonii* by its longer, narrower conidia and its conidiogenous cells lacking percurrent proliferations.

TYPE: China, Sichuan Province: national forest park of Longchi, on dead stems of unidentified broad-leaved tree, 17 Apr 2012, J.W. Xia (**Holotype**, HSAUP H6246-1; **isotype**, HMAS 243459).

ETYMOLOGY: in reference to the type locality.

Colonies on natural substrate effuse, pale brown to brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of septate, pale brown, smooth, 2–5 µm wide hyphae. Conidiophores distinct, single, erect,

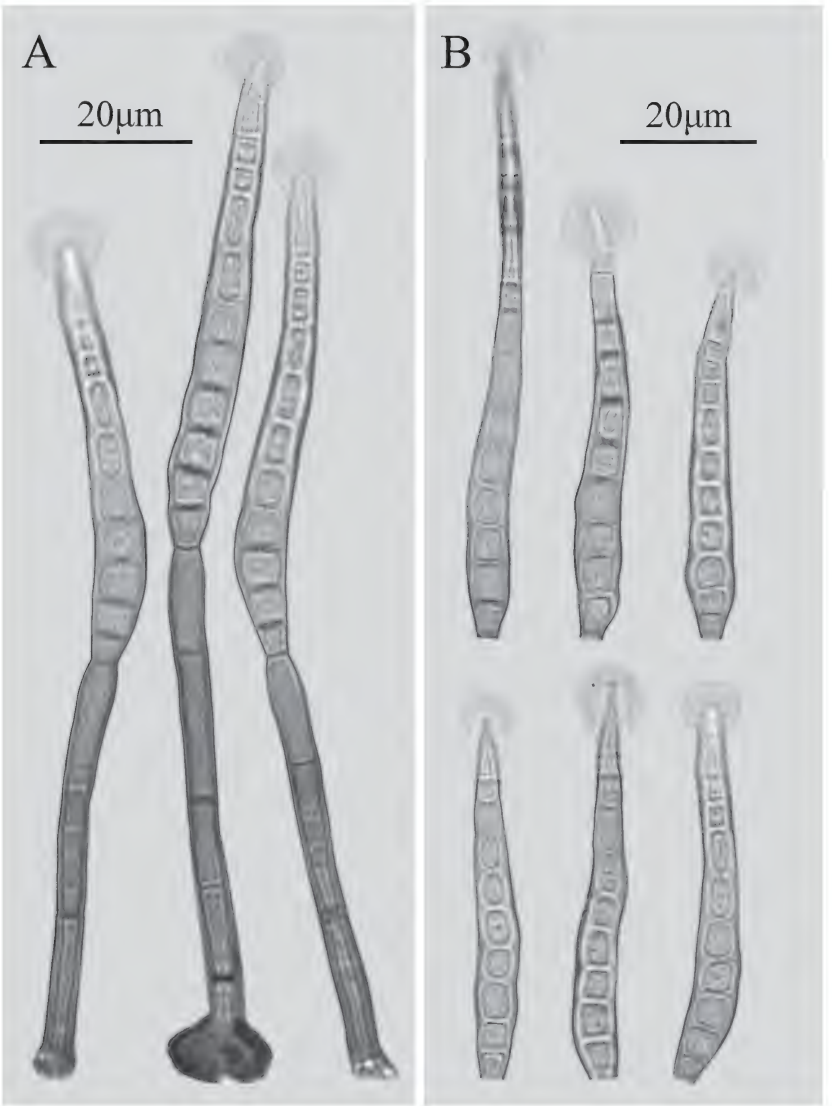

Fig. 2. *Ellisembia longchiensis* (holotype, HSAUP H6246-1).
A. Conidiophores with conidia. B. Conidia.

straight or slightly flexuous, cylindrical, smooth, thick-walled, pale brown to brown, 2–4-septate, 55–75 × 3.5–5 μm. Conidiogenous cells monoblastic, integrated, terminal, pale brown to brown, 13.5–15 × 3.5–5 μm. Conidial

secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or slightly curved, obclavate, smooth-walled, pale brown to brown, paler towards the apex where it is hyaline, 9–12-distoseptate, 55–86 µm long, 6–7 µm thick in the broadest part, 3–4.5 µm wide at the truncate base, with a small, globose, mucilaginous apical sheath.

Comments – *Ellisembia longchiensis* is closely related to *E. mucicola* W.P. Wu and *E. suttonii* W.P. Wu. *Ellisembia mucicola* differs by its shorter, wider, ellipsoid to fusiform conidia (42–52 × 2.5–4 µm, 8–9-septate); and *E. suttonii* differs by its shorter, wider conidia (45–52 × 9–12 µm, 10–12-septate) and its percurrently proliferating conidiogenous cells (Wu & Zhuang 2005).

Acknowledgments

The authors express gratitude to Dr. Eric H.C. McKenzie and Dr. Bryce Kendrick for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.47

Volume 130, pp. 47–56 *January–March 2015*

Fistulina subhepatica sp. nov. from China inferred from morphological and sequence analyses

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Abstract — *Fistulina subhepatica* sp. nov. is described from Yunnan Province, southwestern China. The main characters of *F. subhepatica* are its pileate basidiomata with rose to reddish brown pileal surface and white pore surface, individual and easily separable pores (6–9 per mm), a monomitic hyphal structure with clamped generative hyphae, and ellipsoid basidiospores (4–6 × 3–4 µm). Phylogenetic analysis inferred from the combined ITS (internal transcribed spacer region) and nLSU (the nuclear large subunit ribosomal RNA) dataset support *F. subhepatica* as a distinct new species in *Fistulina*.

Key words — *Agaricales*, *Agaricomycetes*, *Fistulinaceae*, phylogeny, rDNA, taxonomy

Introduction

Fistulina Bull. was established by Bulliard (1790: t.464) and typified by *F. buglossoides* Bull. [= *F. hepatica* (Schaeff.) With.]. It is a small but cosmopolitan genus and causes a brown rot of hardwoods. Morphologically, the genus is characterized by an annual growth habit, reddish to brown pileus surface, separate but closely packed tubes, a monomitic hyphal system, and ovoid basidiospores (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Ryvarden & Gilbertson 1993).

Previous multi-gene phylogenetic studies showed that *Fistulina* fell into the euagarics clade and was closely related to *Schizophyllum* Fr., *Auriculariopsis* Maire, and *Porodisculus* Murrill (Binder et al. 2005, Bodensteiner et al. 2004).

Taxonomic and phylogenetic studies focusing on brown-rot fungi in China have been carried out recently, and some new species have been described (Cui 2013, Cui & Dai 2013, Cui & Li 2012, Cui et al. 2011, Li & Cui 2013a, Li et al. 2013, Shen et al. 2014, Zhou & Dai 2012, Zhou & Wei 2012). As a continuation of these surveys, one undescribed species of *Fistulina* was discovered based on

morphological characters and phylogenetic analysis of the ITS and nLSU rRNA gene regions.

Table 1. List of species, vouchers, and GenBank accession numbers for sequences used in phylogenetic analysis. New sequences are indicated by bold font.

Species	Voucher	GenBank accession no.	
		nLSU	ITS
Boletus satanas Lenz	Bs 2	AF336242	DQ534567
	TDB 1000c	DQ533973	
Coniophora olivacea (Fr.) P. Karst.	402	AF098376	
Favolaschia andina Singer	KG 0025	HM246679	HM246678
F. calocera R. Heim	PDD 70689	AY572006	
	PDD 71528	AY572007	
	SR.KEN. 346	AF261417	
F. cf. *calocera*	JM 98/372	AF261419	
F. peziziformis (Berk. & M.A. Curtis) Kuntze	PDD 67440	AY572008	
Favolaschia sp.	KG 0027	HM246682	HM246681
Fistulina antarctica	REG 550	AY571002	
	CBS 701.85	AY293181	DQ486702
	CIEFAP 115	AY571003	
F. hepatica	DSH 93-183	AY293182	
	REG 593	AY571004	AY571038
F. pallida	CBS 508.63	AY571005	AY571039
F. subhepatica	**Dai 11714**	**KJ925057**	
	Dai 12416	**KJ925053**	**KJ925058**
	Cui 11130	**KJ925054**	**KJ925059**
	Dai 13216	**KJ925055**	**KJ925060**
	Dai 13244 (HT)	**KJ925056**	**KJ925061**
Jaapia argillacea	CBS 252.74	GU187581	GU187524
Mycena epipterygia (Scop.) Gray	GLM 45969	AY207249	
M. galericulata (Scop.) Gray	GLM 45970	AY207251	
	TENN 7495	AF261412	
	RV 87/14.01	AF042636	
M. pura (Pers.) P. Kumm.	CBH 371	FN394630	KF913023
	IS 10/11/2000	FN394634	FN394611
	CBH 367	FN394631	KF913022
	CBH 358	FN394629	FN394608
M. rosea Gramberg	CBH 097	FN394628	FN394556
	TL 12393	FN394641	FN394555
	UP 2	FN394632	FN394550
M. sanguinolenta (Alb. & Schwein.) P. Kumm.	430360	AB512401	AB512311
Panellus edulis Y.C. Dai et al.	HMJAU 7066	GQ219739	GQ219731
	HMJAU 7214	GQ219738	GQ219730

Paxillus involutus (Batsch) Fr.	UBC F16285	EU486436	
Poromycena manipularis (Berk.) R. Heim	JM 98/217	AF261423	
Porodisculus pendulus	HHB-15376-sp	AY293204	
	DAOM 198417	AF261593	
Poromycena sp.	JM 98/128	AF261429	
Suillus cavipes (Opat.) A.H. Sm. & Thiers	TDB-646	AF071535	
Schizophyllum amplum (Lév.) Nakasone	FCUG 1803	AF141873	
S. commune Fr.	NBRC 4928	AB733339	AB733163
	NBRC 6502	AB733340	AB733164
	NBRC 30496	AB733341	AB733165
	NBRC 30749	AB733342	AB733166
S. fasciatum Pat.	CBS 267.60	AF261589	L43385
S. umbrinum Berk.	FL 02.1	AF261590	AF249391

HT = holotype

Materials & methods

Morphological studies

The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Microscopic examinations followed Dai et al. (2010). Sections were studied at magnifications up to 1000× using a Nikon E 80i microscope and phase contrast illumination (Nikon, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with cotton blue and Melzer's reagent. Basidiospores were measured from sections cut from the tubes. In presenting the variation in the basidiospore size, 5% of measurements that were excluded from each end of the range are given in parentheses. In the text, the following abbreviations were used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, CB+ = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios among the specimens studied, n = number of spores measured from given number of specimens. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analysis

The Phire® Plant Direct PCR Kit (Finnzymes, Vantaa, Finland) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen was lysed in 30 µl dilution buffer for DNA extraction. After incubating for 3 min at room temperature, 0.75 µl of the supernatant was used as template for a 30 µl PCR reaction. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 (White et al. 1990), and the nuclear large subunit (nLSU) ribosomal RNA gene regions with the primers LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 34 cycles at 94°C for 40 s, 54°C for 45 s, and 72°C for 1 min, and a final extension of 72°C for 10 min. The

PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 34 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and directly sequenced in Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (Table 1).

Sequences generated for this study were aligned with additional sequences downloaded from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). Prior to phylogenetic analysis, ambiguous positions at the start and the end were deleted and gaps were manually adjusted to optimize alignment. Sequence alignment was deposited at TreeBASE (http://purl.org/phylo/treebase; submission ID 15918).

Phylogenetic analysis was done as in Li & Cui (2013b). Maximum parsimony analysis and Bayesian inference (BI) was applied to the combined ITS and nLSU dataset. *Jaapia argillacea* Bres. was used as outgroup to root trees (Bodensteiner et al. 2004). The maximum parsimony tree was constructed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree (MPT) generated.

MrMODELTEST2.3 (Nylander 2004, Posada & Crandall 1998) was used to determine the best-fit evolution model for BI. BI was calculated with MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) according to the calculated model. Four Markov chains were run for 2 runs from random starting trees for 1.5 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

Results

The combined ITS and nLSU dataset include sequences from 49 fungal collections. The dataset has an aligned length of 1388 characters including gaps, of which 812 characters are constant, 135 are parsimony-uninformative, and 441 are parsimony-informative. Maximum parsimony analysis yielded 9 equally parsimonious trees (Length = 1373, CI = 0.650, RI = 0.867, RC = 0.564, HI = 0.350), and a strict consensus tree of these trees is shown in Fig. 1. The best model for the combined ITS and nLSU partition is a GTR+I+G model. BI produced a topology similar to that from MP analysis with an average standard deviation of split frequencies = 0.007406.

In the phylogenetic tree (Fig. 1), the unknown Chinese *Fistulina* collections grouped in a well-supported lineage (MP = 88%, BPP = 1.00), which had a

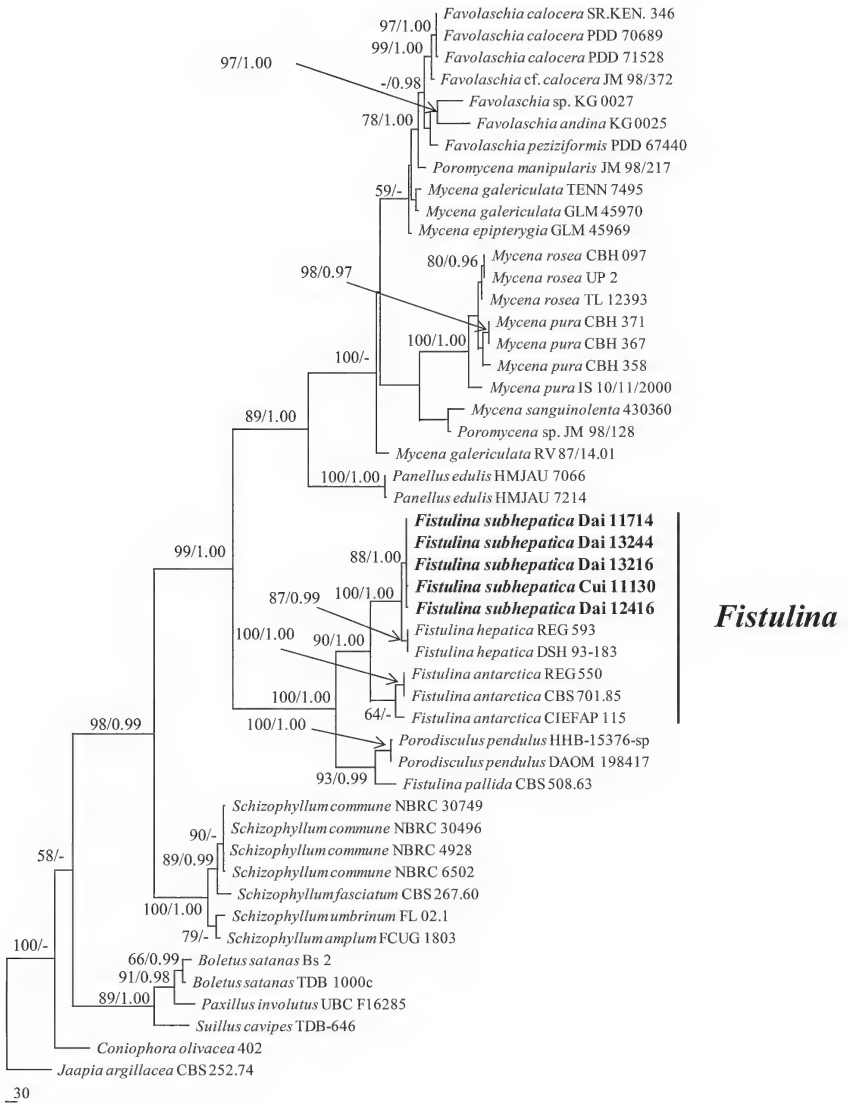

FIGURE 1. The phylogenetic position of *Fistulina subhepatica* based on ITS+nLSU sequence data. Topology is from maximum parsimony analysis. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.95 are indicated at the nodes.

sister relationship with *F. hepatica* [= *F. buglossoides*, the generic type]. The new species is described on the next page.

FIGURE 2. *Fistulina subhepatica* (holotype), basidiomata in situ. Scale bar = 2 cm.

Taxonomy

Fistulina subhepatica B.K. Cui & J. Song, **sp. nov.** FIGS. 2–3

MYCOBANK MB 809111

Differs from *Fistulina hepatica* by its thin- to slightly thick-walled tramal generative hyphae with clamp connections and larger basidiospores.

TYPE: China, Yunnan Province, Jingdong County, Ailaoshan Nature Reserve, on dead tree of *Lithocarpus* (*Fagaceae*), 12.VII.2013, Dai 13244 (**holotype**, BJFC 014732; GenBank KJ925056, KJ925061).

ETYMOLOGY: *subhepatica* (Lat.): refers to the morphological similarity to *Fistulina hepatica*.

FRUITBODY — Basidiomata annual, pileate, fleshy, and readily exuding a reddish blood-like sap when squeezed or bruised when fresh, leathery when dry. Pileus sessile to laterally substipitate, dimidiate to reniform or subcircular, projecting up to 25 cm, 30 cm wide, and 6 cm thick at base. Pileal surface rose to reddish brown when fresh, becoming fuscous to black upon drying, faintly radially furrowed when fresh; margin acute, concolorous. Pore surface white when fresh, turning darker when bruised and becoming cinnamon-buff to reddish brown when dry; pores 6–9 per mm, consisting of individual, crowed but easily separable tubes. Context clay-buff to black, leathery when dry, up to 5 cm thick. Tubes concolorous with pore surface, leathery, up to 1 cm long.

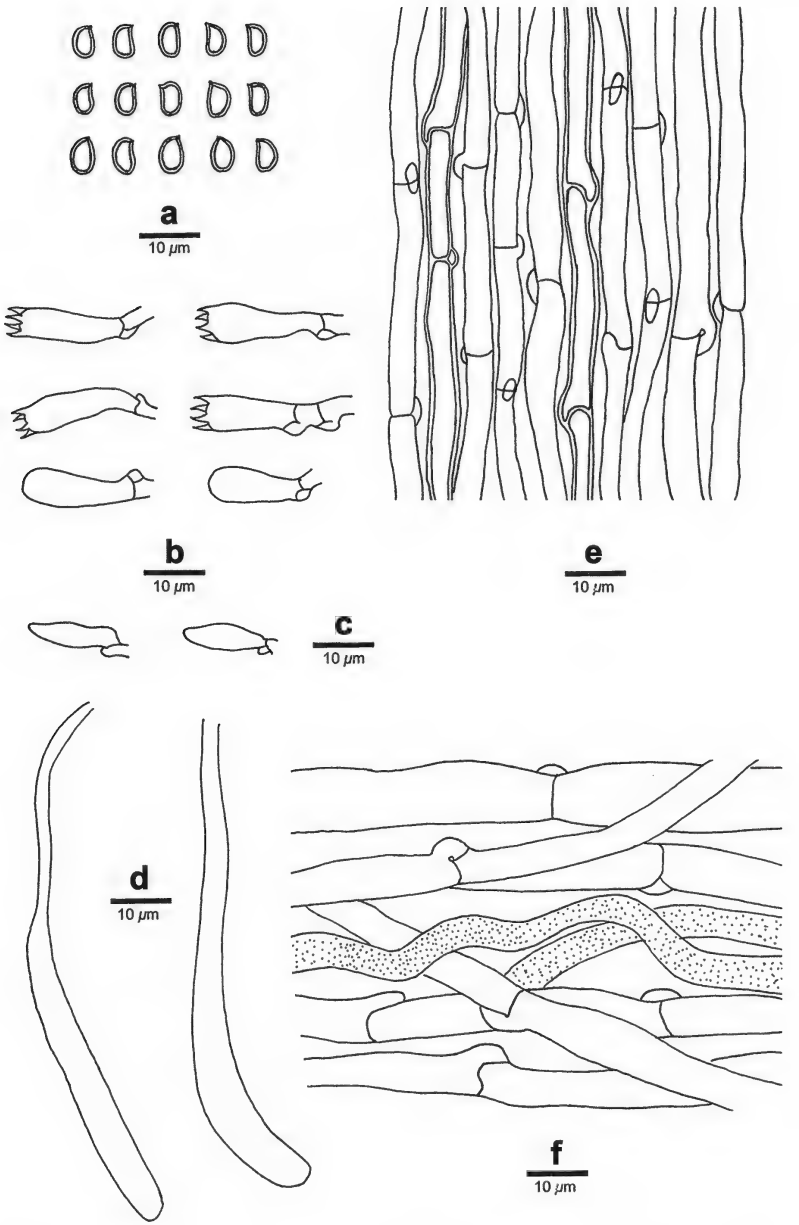

FIGURE 3. *Fistulina subhepatica* (holotype). a: Basidiospores. b: Basidia and basidioles. c: Cystidioles. d: Cystidial elements from dissepiment edges. e: Hyphae from trama. f: Hyphae from context. Scale bars = 10 μm.

Hyphal structure — Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–, more or less dissolving in KOH.

Context — Generative hyphae hyaline, thin-walled, rarely branched, interwoven, 5–12 µm in diam, but with inflated portions up to 18 um, gloeoplerous hyphae present.

Tubes — Generative hyphae hyaline, thin- to slightly thick-walled, rarely branched, parallel along the tubes and densely in sections from dried specimens, 3.5–8 µm in diam. Basidia clavate with four sterigmata and a basal clamp connection, 15–32 × 5–7 µm; basidioles in shape similar to basidia, but slightly smaller. Cystidial elements present in the dissepimental edges, hyaline, thin-walled, 75–90 × 5–7 µm.

Spores — Basidiospores ellipsoid, hyaline, thick-walled, smooth, IKI–, CB+, 4–6 × 3–4(–4.5) µm, L = 4.82 µm, W = 3.37 µm, Q = 1.36–1.52 (n = 150/5).

Additional specimens examined — **CHINA. Yunnan Province**, Lincang, Xiaodaohe Forest Farm, on dead tree of *Castanopsis* (*Fagaceae*), 10.VII.2013, Dai 13216 (BJFC 014706; GenBank KJ925055, KJ925060); Nanhua County, Dazhongshan Nature Reserve, on fallen angiosperm trunk, 15.VII.2013, Cui 11130 (BJFC 015245; GenBank KJ925054, KJ925059); Chuxiong, Zixishan Forest Park, on dead tree of *Lithocarpus*, 28.VIII.2010, Dai 11714 (BJFC 008827; GenBank KJ925057); 11.VI.2011, Dai 12416 (BJFC 010696; GenBank KJ925053, KJ925058).

Other specimen examined — *Fistulina hepatica*. **FINLAND. Vantaa**, Tammosto Nature Reserve, on dead tree of *Quercus* (*Fagaceae*), 8.IX.2012, Dai 12874 (BJFC 013153).

Discussion

The previous reports of *Fistulina hepatica* in China (Dai 2012, Dai et al. 2011, Wang et al. 2011, Yuan & Dai 2008) were from identifications based only on morphological characters. According to the current phylogenetic analysis and more careful morphological examinations, these Chinese samples are newly described as *F. subhepatica*. Macroscopically, *F. hepatica* is quite similar to *F. subhepatica* in its pinkish brown to more reddish or purplish brown pileal surface, white pore surface when fresh, and individual pores; microscopically, however, *F. hepatica* differs from *F. subhepatica* by having smaller basidiospores, simple septate and thin-walled trama generative hyphae (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Ryvarden & Gilbertson 1993). Moreover, *F. hepatica* forms a distinct lineage separate from *F. subhepatica* (Fig. 1). In addition, *F. hepatica* is widely distributed in temperate Europe and grows mainly on *Quercus* and *Castanea*, whereas *F. subhepatica* is found in subtropical China, occurring mostly on *Castanopsis* and *Lithocarpus*. We did not find *F. hepatica* on *Quercus* in northern China, although the big and old oak trees are suitable for the fungus.

Fistulina antarctica Speg., closely related to *F. subhepatica* in the phylogenetic analysis, differs morphologically from *F. subhepatica* by its longer basidiospores (5–7 × 3–4 µm, Spegazzini 1887).

Fistulina pallida Berk. & Ravenel resembles *F. subhepatica* in its brownish pore surface and individual pores; however, it produces smaller basidiomata and wood-brown pileus surface (Gilbertson & Ryvarden 1986). Phylogenetically, *F. pallida* falls outside the "core *Fistulina* clade" and groups with *Porodisculus pendulus* (Fr.) Murrill.

Acknowledgments

The authors are grateful to Prof. Yu-Cheng Dai (BJFC, China) for collecting specimens and improving the text. We express our gratitude to Drs. Li-Wei Zhou (China) and Tatiana B. Gibertoni (Brazil), who reviewed the manuscript. The research was financed by Beijing Higher Education Young Elite Teacher Project (YETP0774) and the National Natural Science Foundation of China (Project No. 31170018).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.57

Volume 130, pp. 57–59 *January–March 2015*

Antherospora sukhomlyniae, a new species of smut fungi on *Hyacinthella* in Crimea (Ukraine)

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Abstract — *Antherospora sukhomlyniae,* a new species of smut fungus parasitic on *Hyacinthella* sp. in Crimea (Ukraine), is described and illustrated.

Key words — anther smut, biodiversity, mycobiota, *Ustilaginomycetes*

Introduction

During a collecting trip for smut fungi in Crimea (Ukraine), carried out by the author in 2009, several specimens of smut fungi were collected. Based on the study of the specimen from anthers of *Hyacinthella* sp. (*Asparagaceae*), I propose here a new smut fungus, *Antherospora sukhomlyniae,* that was collected in Karadag reserve in the southeastern part of the Crimean peninsula.

Materials & methods

Sorus and spore characteristics were studied using dried herbarium specimens. Spores were dispersed in a droplet of lactic acid on a microscope slide, covered with a cover glass, gently heated to boiling point to rehydrate the spores, cooled, and then examined using a Carl Zeiss Axiostar light microscope (LM) at 1000× magnification. For scanning electron microscopy (SEM), spores were attached to specimen holders by double-sided adhesive tape and coated with gold. The surface structure of spores was observed at 15 kV and photographed with a scanning electron microscope JEOL JSM-6700F.

The holotype specimen is conserved in the Herbarium, Department of Biodiversity and Biotechnology of Fungi, Institute of Evolution, University of Haifa, Israel (HAI).

Taxonomy

Antherospora sukhomlyniae K.G. Savchenko, **sp. nov.** Fig. 1

MycoBank 808616

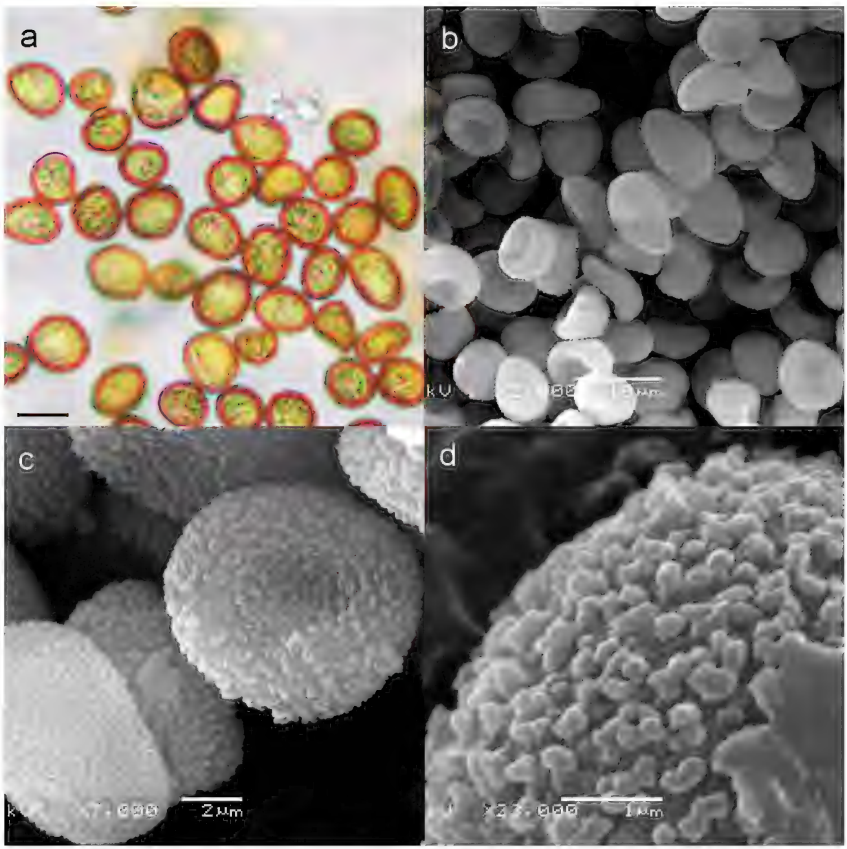

FIGURE 1. *Antherospora sukhomlyniae* (holotype) in flowers of *Hyacinthella* sp.: spores. a = LM; b–d = SEM. Bars: a, b = 10 µm, c = 2 µm, d = 1 µm.

Differs from all other *Antherospora* species by its host specialization on *Hyacinthella* and from *A. albucae* and *A. urgineae* by its smaller spores, from *A. eucomis* by its irregularly shaped spores, and from *A. tourneuxii* by its thicker spore walls.

TYPE: Ukraine, Autonomous Republic of Crimea, Karadag reserve, in flowers of *Hyacinthella* sp., 5.V.2009, leg. K.G. Savchenko (**Holotype** HAI 6521).

ETYMOLOGY: named in honor of the Ukrainian mycologist Maryna M. Sukhomlyn.

Parasitic on *Hyacinthella*. Sori in all inner floral organs of deformed and swollen flowers, producing blackish brown, powdery mass of spores, for a long time enclosed by the outermost floral envelopes. Infection systemic, with all flowers of the plant infected. Spores ovoid, ellipsoidal, subglobose to irregular, (6.5–)7–10 × 7.5–13(–14) µm, yellowish brown. Spore wall even, ca. 0.8 µm thick, finely densely punctate-verrucose, with finely wavy spore profile.

Distribution – Known only from the type locality in Crimea, on *Hyacinthella* sp.

Comments – Previous studies have demonstrated that while all species of *Antherospora* attack hosts in the *Asparagaceae,* they display a high level of specificity, with each species restricted to host plants from a single genus, or in some cases, from a single species (Bauer et al. 2008, Piątek et al. 2011, 2013). As no *Antherospora* species has been reported on *Hyacinthella,* I propose a new species here.

Antherospora sukhomlyniae belongs to the group of *Antherospora* species with sori destroying all inner floral organs of infected flowers (Vánky 2012), which includes *A. albucae* (Syd. & P. Syd.) R. Bauer et al., *A. eucomis* Vánky, *A. tourneuxii* (A.A. Fisch. Waldh.) R. Bauer et al., and *A. urgineae* (Maire) R. Bauer et al. However, these species can be distinguished from *A. sukhomlyniae:* A. *albucae* by its larger spores (7–14.5 × 9.5–22.5 µm; Vánky 2012); *A. urgineae* by its larger spores (7–12 × 9.5–17.5 µm; Vánky 2012); *A. eucomis* by its regularly shaped spores with thinner spore walls (0.5 µm; Vánky 2009); and *A. tourneuxii* by its thinner spore walls (0.5 µm; Vánky 2012).

Acknowledgments

The author thanks Lori M. Carris and Vasyl P. Heluta for peer-reviewing the manuscript, Shaun Pennycook for useful corrections, and Vitalii Sapsai for help with the SEM microscopy.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.61

Volume 130, pp. 61–68 *January–March 2015*

Tuber xanthomonosporum, a new *Paradoxa*-like species from China

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Abstract — A new species, *Tuber xanthomonosporum*, is described based on specimens collected under *Pinus yunnanensis* in Panzhihua, Sichuan Province, China. *Tuber xanthomonosporum* invariably has only one spore per ascus. It can be distinguished from the three other Chinese *Paradoxa*-like species — *T. gigantosporum*, *T. sinomonosporum*, and *T. glabrum* — by its whitish to yellow-brownish gleba, two-layered peridium, and spiky cystidia. Molecular analysis also supports *T. xanthomonosporum* as a unique species.

Key words — taxonomy, *Pezizales*, Yunnan

Introduction

The genus *Paradoxa* was erected by Mattirolo in 1935 in the *Tuberaceae* to accommodate *Paradoxa monospora* Mattir., a species which, invariably, has one-spored asci (Montecchi & Sarasini 2000; Læssøe & Hansen 2007). For the following 50 years the genus remained monotypic until Wang & Hu (2008) transferred *Tuber gigantosporum* into *Paradoxa*. Wang & Li (1991) originally described *T. gigantosporum* based on a single specimen collected from Huidong County, Sichuan, China, in 1988. The species was characterized by its extremely large elliptical ascospores and one-spored asci, a feature that was eventually recognized as being important when a second *T. gigantosporum*

collection was made in Yongren County, Yunnan, China, in 2002. Since then three other *Paradoxa*-like species have been reported: *Tuber* sp. 1 (Kinoshita et al. 2011), *Paradoxa sinensis* (Fan et al. 2012), and *Tuber glabrum* (Fan et al. 2014). Recent molecular phylogenetic analysis of truffle species supported *Paradoxa* within *Tuber* (Kinoshita et al. 2011, Fan et al. 2013), and subsequently Fan et al. (2014) transferred *P. sinensis* into *Tuber* with the replacement name *T. sinomonosporum*.

In 2012 during a survey in Panzhihua in the southwestern corner of Sichuan, China, we found truffles that invariably had one-spored asci. Here we describe this *Paradoxa*-like truffle as a new species of *Tuber* and briefly review the molecular relationships of truffle species with single-spored asci.

Table 1: Origin of the fungal ITS sequences. (New sequences are indicated in bold font.)

Taxon	Voucher or code	Geographical origin	GenBank no.
Choiromyces meandriformis	isolate RH691	USA	HM485330
	isolate GB285	USA	HM485331
Gymnohydnotrya australiana	isolate OSC130601	USA	JQ925629
Tuber sp. 1	isolate K201	Japan	AB553344
Tuber sp. 2	isolate K215	Japan	AB553356
T. borchii	clone Bo6	Italy	AF106890
	strain Z43	Italy	AF250291
	Unknown	France	AF132505
T. canaliculatum	JT12670	USA	GQ221455
	JT23942	USA	GQ221456
T. excavatum	TE-M	Poland	KC330228
	isolate zb3281_215	Germany	HM152020
	TE-WD	Poland	KC330227
T. foetidum	isolate B-2452	Hungary	AJ557543
	isolate B-2489	Hungary	AJ557544
T. fulgens	isolate y466	Germany	HM152014
	isolate zb3386_202	Germany	HM152024
	isolate M2435	USA	HM485358
T. glabrum		China	KF002731
		China	KF002732
T. indicum	strain T39	China	JQ638997
	strain T47	China	JQ639005
	strain T49	China	JQ639007
T. latisporum	HKAS 44315	China	DQ898183
	HKAS 42380	China	DQ898184

T. liui	HKAS 48269	China	DQ898182
T. macrosporum	isolate JT19458	USA	HM485372
	isolate S7510	Germany	JF926121
T. maculatum	clone Mac1	Italy	AF106889
	1967	New Zealand	EU753269
T. melanosporum	strain1015	Israel	AF167096
	LHB-mic-Tmel-Qc-09-1	Spain	GU810153
	LHB-mic-Tmel-Qr-09-1	Spain	GU810152
T. panzhihuanense	strain DXJ260	China	JQ978644
	strain DXJ276	China	JQ978650
T. pseudoexcavatum	isolate CJ408	USA	HM485381
	strain E	China	JQ638958
	strain Q	China	JQ638982
	strain T48	China	JQ639006
T. rufum	1798	Italy	EF362473
	1447	Italy	EF362477
T. sinomonosporum	BJTCFAN150	China	KF002729
T. umbilicatum	HKAS48267	China	GU979032
	HKAS44316	China	GU979031
	isolate T104	China	FJ797879
	isolate T117	China	FJ797880
T. xanthomonosporum	**YAAS L3185**	**China**	**KJ162154**
	YAAS L3186	**China**	**KJ162155**
	YAAS L3187	**China**	**KJ162156**
T. zhongdianense	HKAS 45388B	China	DQ898186
	wang0299	China	DQ898187

Materials & methods

Morphological studies

Specimens of a truffle with asci containing a single spore were collected from Panzhihua, Sichuan, China, in 2012. Specimens are deposited in the Herbarium of Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, China (YAAS).

The samples were examined at the Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, China and Sichuan Panzhihua Academy of Agriculture and Forestry, Panzhihua, Sichuan, China. Macroscopic characters were described from fresh specimens and microscopic features described from dried specimens following the methods of Yang & Zhang (2003). Photographs were made under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto double-sided tape and mounted directly on a SEM stub. They were then coated with gold-palladium and examined and photographed with a JEOL, JMS-5600LV SEM.

DNA amplification & sequence analysis

DNA was extracted using CTAB (Doyle 1987) modified by adding 200 µL 5M potassium acetate after 4 × CTAB treatments. The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS-rDNA region. PCR reaction solution and cycling parameters used by Chen & Liu (2007) were adopted. The amplification products were electrophoresed on a 1% agarose gel and purified with Sangon's purification kit. Sequencing was performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL automatic sequencer.

In order to determine whether the specimens represented a new species, we compared our *Tuber xanthomonosporum* ITS-rDNA sequences with 48 ITS-rDNA sequences downloaded from NCBI. An ITS-rDNA sequence of *Gymnohydnotrya australiana* B.C. Zhang & Minter was also included as outgroup. Sequence alignment and phylogenetic analysis protocols followed Chen & Liu (2007).

Results

Phylogenetic analysis

A total of 52 ITS sequences were included in a phylogenetic analysis (Table 1, Plate 2). Of the 712 total characters analyzed, 248 are constant, 51 parsimony-uninformative, and 413 parsimony-informative (L = 1979, CI = 0.481, RI = 0.812, RC = 0.390). ITS sequence analysis revealed that the three *Paradoxa*-like species (*Tuber* sp. 1, *T. sinomonosporum*, *T. glabrum*) form a well-supported subclade (bootstrap support = 98%) within Clade I, which also included *T. macrosporum*, *T. canaliculatum,* and *Tuber* sp. 2. In contrast, *T. xanthomonosporum* does not cluster with the other *Paradoxa*-like species but forms a new Subclade I in Clade II (with low bootstrap support) alongside Subclade II (/melanosporum) and Subclade III (/rufum).

Taxonomy

Tuber xanthomonosporum Qing & Y. Wang, **sp. nov.** Plate 1

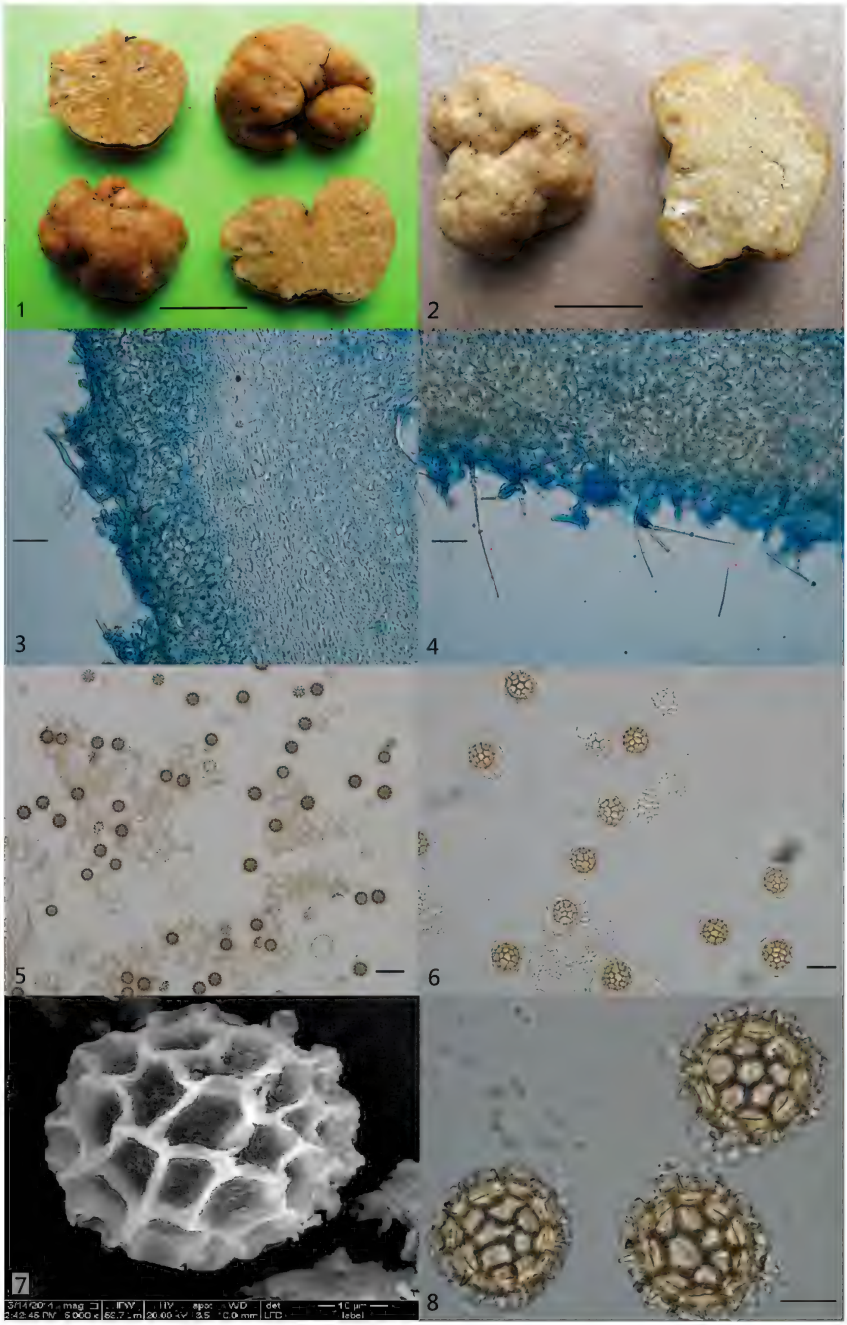
MycoBank MB 807668

Differs from other *Tuber* species with single-spored asci by its whitish to yellow-brownish gleba and two-layered peridium with spiky cystidia.

Type: China, Sichuan Province, Panzhihua, 26°29′N 102°01′E, under *Pinus yunnanensis* Franch., 15 Nov. 2012: Y. Qing Pan1201 (**Holotype**, YAAS L3185; GenBank KJ162154).

Etymology: *xanthomonosporum* from Latin refers to the yellow brownish gleba and single spored asci.

Plate 1. *Tuber xanthomonosporum* (YAAS L3185, holotype): **1**, **2**. Fresh ascomata showing surface features and cut sections. **3**. Cross section of peridium showing two layers of pseudo-parenchymatous and interwoven tissue with some cystidia. **4**. Cross section of outer layer of peridium showing two kinds of cystidia: tall and tapered (arrows), and short and obtuse (arrow head). **5–7**. Ascospores and asci (invariably with only one spore). **8**. Ascospores showing ornamentations. Scale bars: 1, 2 = 1 cm; 3, 8 = 20 µm; 4, 7 = 50 µm; 5 = 100 µm; 6 = 40 µm.


1
2
3
4
5
6
7
8

Ascomata subglobose or irregular and much lobed with deep furrows, firm and a little rubbery, whitish to yellow-brownish, up to 3.0 cm broad, rough with hairs. Odor slightly garlicky when mature. Peridium 160–200 µm thick, composed of two layers; the outer layer complex, 50–70 µm thick, pseudoparenchymatous, composed of irregular globose to rectangular cells, 19–32 × 13–15 µm diam and thin-walled; the outmost cells giving rise at the surface to cystidia of two types; one tall, thin, tapered, reaching 50–90 × 2–4 µm and sometimes with a small teat on the wall, hyaline; the other short, obtuse, 1–2-septate, 15–20 × 4–5 µm; the inner layer 110–130 µm thick, composed of mainly interwoven, colourless hyphae of 2–4 µm diam with some big cells of 2–4 µm diam, that gradually merge into the gleba tissue. Gleba solid, whitish when young, becoming yellow-brownish at maturity, marbled with distinct, white and meandering veins, merging at many points with the peridium. Asci 60 × 70 µm, globose to subglobose, sessile, slightly thick walled 2–3 µm thick, invariably 1-spored and randomly dispersed in glebal tissue. Ascospores globose, 41–43 × 43–46 µm; spore walls 2.5 µm thick, brown at maturity, ornamented with a regular alveolate-reticulum, 2–3 µm deep, constituted of mostly hexagonal meshes 8–15 × (5–)7–13 µm, 4–5 across the spore width.

Ecology & distribution — Hypogeous, solitary or in groups in calcareous soils with pH 6.8–7.6 under *Pinus yunnanensis* and forming ectomycorrhizae with its roots, alt. 1500–2000 m, fruiting from autumn to winter. Known only from Sichuan, China.

Additional specimens examined: **CHINA, Sichuan Province, Panzhihua,** 26°29′N 102°01′E, under *Pinus yunnanensis,* 15 Nov. 2012, C.Y. Liu Pan-No-10 (YAAS L3186; GenBank KJ162155); C.Y. Liu Pan-No-40 (YAAS L3187; GenBank KJ162156).

Discussion

The new *Tuber* species, *T. xanthomonosporum* from Sichuan, produces only one spore per ascus, the diagnostic character of the now defunct genus, *Paradoxa* (Mattirolo 1935; Fan et al. 2014). Recent phylogenetic studies of *Paradoxa*-like species (Kinoshita et al. 2011; Fan et al. 2014) found that they group with *Tuber* species. Our phylogenetic analysis groups *T. xanthomonosporum* with *Tuber* species but not with other *Paradoxa*-like species, despite sharing the common character of having only one spore per ascus. Our work therefore supports the conclusion by Fan et al. (2014) that *Paradoxa*-like truffle species do not form a monophyletic group. While *Tuber sinomonosporum* [≡ *P. sinensis*], *T. glabrum,* and *T.* sp. 1 (the Japanese *Paradoxa*-like new species) do group together, they also group with *T. macrosporum* and *T. canaliculatum* in Clade I (/macrosporum). These species in Clade I share the same morphological feature of having red-brown gleba. *Tuber xanthomonosporum* groups in Clade II with the /melanosporum and /rufum subclades but with very low bootstrap support (<50%). Morphologically, *T. xanthomonosporum* differs greatly from

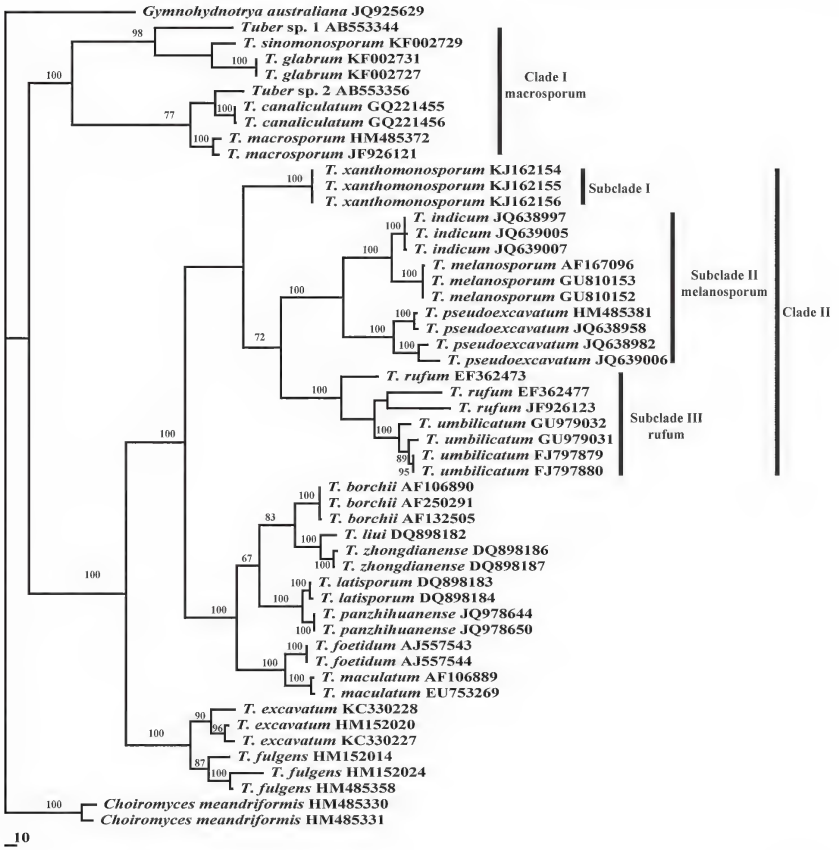


Plate 2. One of eight most parsimonious trees constructed with ITS sequences of *Tuber xanthomonosporum* and related species. MP Bootstrap values greater than 50% are indicated at nodes.

the species in these two subclades in its whitish to yellow-brownish gleba and reticulate spores. Given the few species and low support for the species now placed in Clade II, the phylogenetic relationships need further study with more samples and gene loci.

Acknowledgements

We are grateful to Dr. Ian Hall and Dr. A. Zambonelli for reviewing the paper. We also thank Prof. Fan Li and Mr. H.K. Xiong for providing *Paradoxa*-like specimens for our study. This study was supported financially by the National Science Foundation of China (No.31160010) and the Science and technology support program of Sichuan Province (No.2012NZ0003 and No.2013NZ0029).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.69

Volume 130, pp. 69–72 *January–March 2015*

Distance1D – a protein profile analytical program designed for fungal taxonomy

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Abstract —Taxonomic analysis of macromycete fruiting bodies is a challenging task that utilizes morphological, biochemical, and molecular methods. Many biochemical and molecular methods have been developed to test or confirm identifications or phylogenetic positions independently of morphological data. SDS electrophoresis has been shown to be a good biochemical method for protein separation. Although protein profiles can be analyzed by commercially available software, there is no software designed specifically for fungal taxonomic research. We have developed an open source portable program that uses protein profiles of fungal fruiting bodies to calculate relative differences between species for use in generating to generate more accurate phylogenetic trees.

Key words — biochemistry, fungi, Perl, proteome

Introduction

The challenging task of macromycete taxonomy uses morphological, biochemical, ecological, and molecular data (Guarro et al. 1999, Blackwell et al. 2006, Korabecna 2007, Lutzoni & Vilgalys 1995). Sodium dodecyl sulfate (SDS) electrophoresis is a good biochemical method for protein separation and biomarker discovery, which when applied to the proteomes of fungal fruiting bodies gives a good number of separated proteins, revealing small (or sometimes large) differences in protein expression (Materić 2012). The method, which is robust and reliable, could serve as an independent taxonomic tool for fungi (Guarro et al. 1999, Tyrrell 1969).

The aim of this work was to develop a user-friendly open source program using electrophoresis data for fungal taxonomy. Such a program would compare and calculate relative differences between taxa based on results from SDS gel electrophoresis and similar techniques (e.g., western blot, zymography).

Materials & methods

We sampled and identified 21 fruiting bodies representing differently related species. Proteins were separated according to the method described in Materić (2012), and protein profiles were obtained for each species. The quality of protein profiles depends upon many factors during sample homogenization, protein extraction and electrophoresis, and an optimized protocol can be found in Materić et al. (2012). In this work we have chosen to show the results of only seven species: however, more detailed dendrograms are available in Materić (2012).

Protein profile data are stored in .csv format and examples of files can be found along with the source-code in Materić (2013). Protein profile files include two sets of data: (1) molecular weights (MW) of proteins; and (2) relative abundance. MW and relative abundances can be obtained using specialized software such as TotalLab (Phoretix, Newcastle, United Kingdom). However, abundances should be recalculated as relative abundances with range of 1 (least abundant) to 5 (most abundant). In order to create a distance matrix table, protein profiles are compared by distance1D. The distance matrix tables are transformed into dendrograms using the program package PHYLIP (Felsenstein 2002).

Results & discussion

Protein electrophoresis, which has been widely used in solving taxonomic and systematic problems, produces results that are independent of morphological and molecular data (Guarro et al. 1999, Tyrrell 1969). Thus, it is crucial to have appropriate software that deals with taxonomic issues rather than with pure biochemical properties. Apart from distance1D, there is no such software specifically designed to interpret fungal taxonomy from protein profile data.

Distance1D is a program designed to calculate relative differences between two protein profiles, which could then be used to generate dendrograms (Fig. 1). The program is designed and written specifically for use in fungal taxonomy (mainly for analyzing protein profiles of fungal fruiting bodies), but its usage could be wider. The program is meant to be open source, user-friendly, and portable. Portability results from the program's being written in Perl, which can be run on any operating system. As the program uses the Tk library for generating widgets (Windows-like interface), Perl/Tk should be installed along with Perl.

Our previous work produced good protein profiles of fungal fruiting bodies from SDS gel electrophoresis (Materić 2012) that are stored in separate files (Fig. 1).

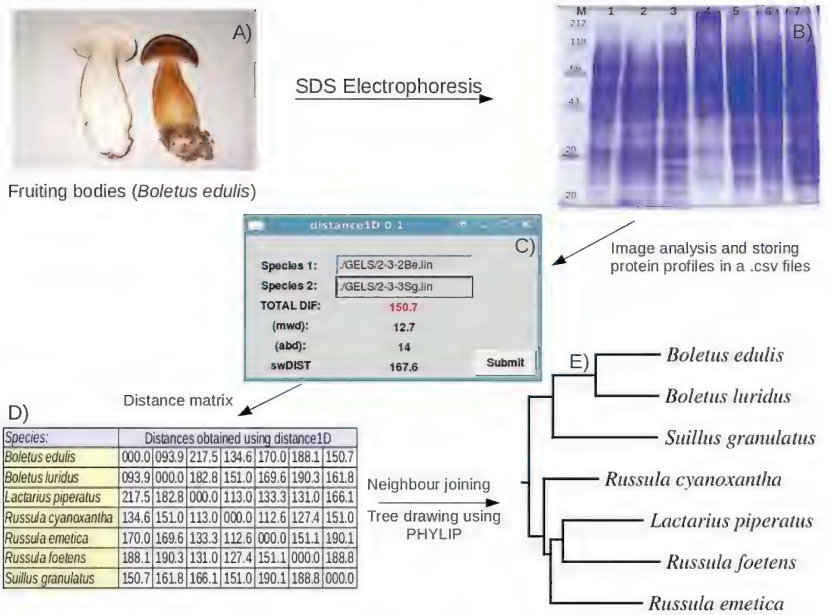
A)
Fruiting bodies (Boletus edulis)
SDS Electrophoresis
M 1 2 3 4 5 6 7
B)
distance1D 0.1
C)
Species 1: /GELS/2-3-2Be.lin
Species 2: /GELS/2-3-3Sg.lin
TOTAL DIF: 150.7
(mwd): 12.7
(abd): 14
swDIST 167.6
Submit
Image analysis and storing protein profiles in a .csv files
Distance matrix
D)
Species: | Distances obtained using distance1D
Boletus edulis | 000.0 | 093.9 | 217.5 | 134.6 | 170.0 | 188.1 | 150.7
Boletus luridus | 093.9 | 000.0 | 182.8 | 151.0 | 169.6 | 190.3 | 161.8
Lactarius piperatus | 217.5 | 182.8 | 000.0 | 113.0 | 133.3 | 131.0 | 166.1
Russula cyanoxantha | 134.6 | 151.0 | 113.0 | 000.0 | 112.6 | 127.4 | 151.0
Russula emetica | 170.0 | 169.6 | 133.3 | 112.6 | 000.0 | 151.1 | 190.1
Russula foetens | 188.1 | 190.3 | 131.0 | 127.4 | 151.1 | 000.0 | 188.8
Suillus granulatus | 150.7 | 161.8 | 166.1 | 151.0 | 190.1 | 188.8 | 000.0
Neighbour joining
Tree drawing using PHYLIP
E)
Boletus edulis
Boletus luridus
Suillus granulatus
Russula cyanoxantha
Lactarius piperatus
Russula foetens
Russula emetica

FIGURE 1. Diagram showing stages of an experiment used in order to gain biochemically based phylogenetic trees. A: Fruiting bodies. B: Result of SDS electrophoresis (channels: M = molecular mass marker; 1 = *Boletus luridus*; 2 = *B. edulis*; 3 = *Suillus granulatus*; 4 = *Russula foetens*; 5 = *Lactarius piperatus*; 6 = *R. cyanoxantha*; 7 = *R. emetica*; Materić et al 2012). C: Interface of the program distance1D. D: Distance matrix table generated by the program. E: Phylogenetic tree drawn by PHYLIP program package (similar results were obtained by analyzing ITS II regions of rDNA as control; Materić 2012).

The program as an input takes the paths of two files (two file names) where protein data are stored in .csv format. An algorithm searches for optimal pairing of proteins by looping through the parameters, such as MW sensitivity. After alignment and optimization, the following differences are scored: (1) presence of new protein lines (new protein), (2) molecular weight difference of protein pairs, and (3) abundance difference of protein pairs. The scoring system, which could be adapted according to user requirements, is set up as follows: "10" scored for each new protein line, "1" scored for each 1kDa MW shift, and "2" scored for abundance difference. If DNA/RNA gel data are going to be used, the abundance difference for each fragment should be set the same.

There are several important advantages to using this program: the program generates distances that give accurate phylogenetic trees (more accurate than a single DNA sequence such as rDNA ITS1; Materić 2012); the parameters can be changed to suit particular research; the program can be easily adapted

for use with any protein/DNA/RNA electrophoresis data; and the program is suitable for all operating systems and is open source and free.

Acknowledgments

The authors thank Milan Matavulj (University of Novi Sad, Serbia) and Mirjana Stajic (University of Belgrade, Serbia) for presubmission review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.73

Volume 130, pp. 73–77 *January–March 2015*

Coccomyces prominens sp. nov. (*Rhytismataceae*) on *Rhododendron coeloneurum* in China

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Abstract — A fungus found on leaves of *Rhododendron coeloneurum* from Garze prefecture (Sichuan Province), China, is described as *Coccomyces prominens*. The new species is similar to *C. urceoloides* but differs by its much larger intraepidermal ascomata and the presence of conidiomata. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — foliicolous fungus, morphological character, taxonomy, *Ericaceae*

Introduction

Coccomyces De Not. is the second-largest genus of *Rhytismataceae* (*Rhytismatales*, *Leotiomycetes*, *Ascomycota*), represented by 116 species recorded by Kirk et al. (2008) plus a few recently added. Members of this genus are widely distributed and occur on leaves, twigs, bark, or wood of vascular plants, especially *Ericaceae*, *Fagaceae*, and *Lauraceae* (Sherwood 1980). Twenty-eight *Coccomyces* species have been reported from China (Korf & Zhuang 1985; Lin 1998; Hou et al. 2006, 2007; Jia et al. 2012; Zheng et al. 2012; Wang et al. 2013; Yang et al. 2013) since Teng (1934) first recorded *C. dentatus* (J.C. Schmidt) Sacc. on *Quercus* and *Castanea* and *C. delta* (Kunze) Sacc. on *Lauraceae*.

Here, *Coccomyces prominens* on leaves of *Rhododendron coeloneurum* from Luding county, Garze prefecture, Sichuan Province, China, is described as a new species.

Materials & methods

Mature fruiting bodies were selected from the collected specimens. External shapes, size, color, ascomatal and conidiomatal openings, and zone lines were observed under

a dissecting microscope at 10–50× magnification. After rehydration in water, 10–20 µm thick vertical transverse sections of fruiting bodies were made using a freezing microtome and mounted in 0.7% (w/v) cotton blue in water. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or 0.1% (w/v) cotton blue in phenol glycerin. The colors of internal structures and paraphysis contents were observed in water. Measurements were made using material in 5% KOH and from ca 30 paraphyses, asci, ascospores, conidiogenous cells, and conidia for each specimen. Figures were drawn of the external and internal structures of the fruiting bodies using a microscopic drawing tube. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Coccomyces prominens Y.F. Xu & Y.R. Lin, **sp. nov.** FIGS 1–6

MycoBank MB 808960

Differs from *Coccomyces urceoloides* by its much larger, intraepidermal ascomata with an obvious pre-formed dehiscence mechanism, its wider ascospores enveloped in a gelatinous sheath, and its production of conidiomata.

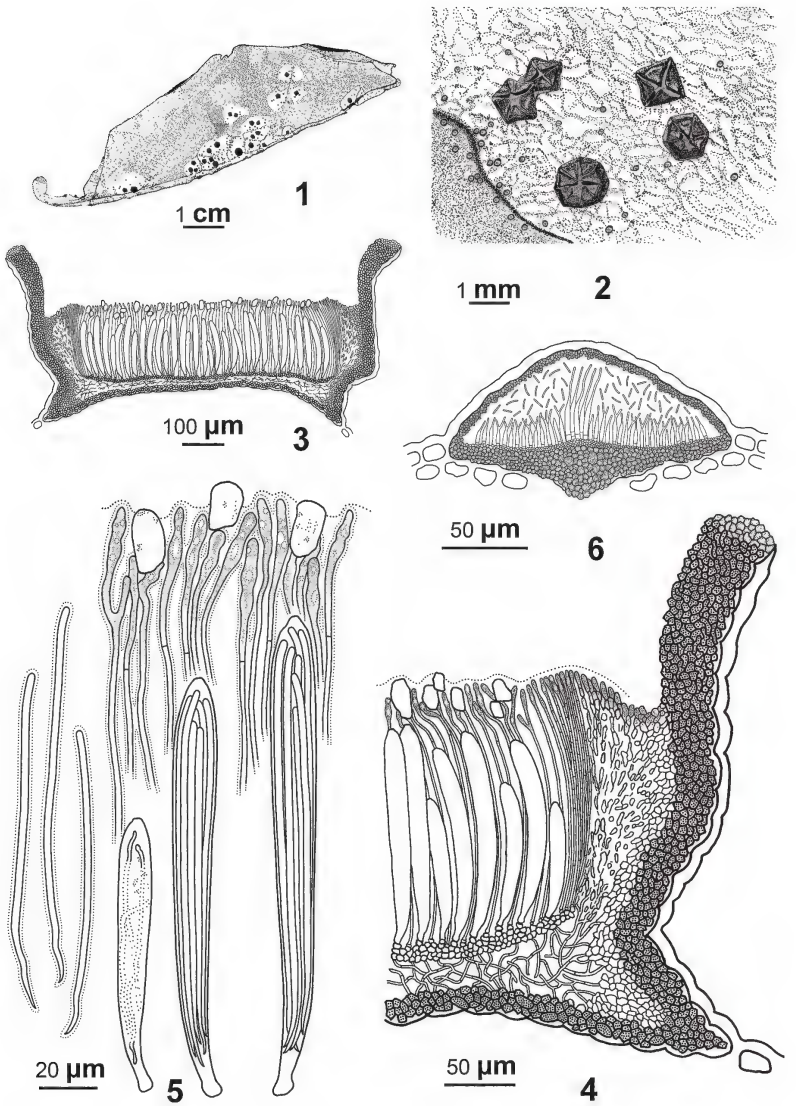
Type: China, Sichuan, Garze prefecture, Luding, Hailuogou, alt. ca 3400m, on fallen leaves of *Rhododendron coeloneurum* Diels (*Ericaceae*), 22 June 2009, Y.G. Liu & Y.R. Lin 2768 (**Holotype**, AAUF 68876).

Etymology: *prominens* (Latin = prominent), referring to the ascomata, which are noticeable on the substratum.

Zone lines usually frequent, brown or dark brown, thin to wide, somewhat diffused, nearly always partly surrounding the bleached spots.

Conidiomata in similar positions to ascomata on the substratum, scattered to clustered, sometimes several coalescent. In surface view, conidiomata 150–220 µm diam., rounded or subrounded, with a clearly marked outline, black-brown, moderately raising the leaf surface, discharging spores through an apical ostiole. In vertical transverse section, conidiomata intraepidermal, lenticular to semicircular. Upper wall 6.5–12 µm thick, almost not attenuating towards the base, composed of disparately sized angular cells. Basal wall 35–50 µm thick in the middle, becoming 13–20 µm thick at periphery, consisting of black-brown, angular and elongate, thick-walled cells 3.5–8 µm diam. Subconidiogenous layer 5–8 µm thick, composed of colorless, thin-walled angular cells 1.2–2 µm diam. Conidiogenous cells 10–14 × 1.5–2.2 µm, cylindrical but tapered towards the apex, hyaline, proliferating sympodially and percurrently. Conidia 4.5–7 × 0.5–0.8 µm, cylindrical, straight or slightly curved to one side, ends rounded or bluntly pointed, hyaline, aseptate, smooth-walled. Trichogynes 35–45 × 2–3 µm, cylindrical, tapering towards the apex, 1–2-septate near the base.

Ascomata epiphyllous, scattered or occasionally coalescent, in subcircular or irregular pale areas with obvious edges. In surface view, ascomata 800–1400



Figs 1–6. *Coccomyces prominens* (ex holotype, AAUF 68876) on *Rhododendron coeloneurum*. 1. A leaf bearing fruiting bodies and zone lines. 2. Conidiomata, ascomata, and a zone line observed under a dissecting microscope. 3. Ascoma in vertical transverse section. 4. Portion of ascoma in vertical transverse section. 5. Paraphyses, asci, and ascospores. 6. Conidioma in vertical transverse section.

μm diam., quadrilateral to octagonal, black-brown to black, not shiny or slightly shiny, edge defined, moderately raising the substrate surface, with an obvious pre-formed dehiscence mechanism, opening by 4–8 radial splits nearly extend to the edge of ascoma. Lips absent. In median vertical transverse section, ascomata intraepidermal. Covering stroma 55–65 μm thick near the opening, decreasing to 25–30 μm thick adjacent to the base, composed of black-brown textura angularis-epidermoidea with thick-walled cells 4–6.5 μm diam., connecting to the basal stroma. Adjacent to the top of the covering stroma, a flimsy structure develops consisting of angular, thin-walled, almost colorless cells staining with cotton blue. Basal stroma 18–22 μm thick, comprised of 2–3(–4) layers of dark brown, angular, thick-walled cells 4–6 μm diam. Excipulum well-developed, 45–60 μm thick, arising from the marginal paraphyses and the internal matrix of stroma, consisting of closely agglutinated septate hyphae 2–5 μm diam., mostly short-celled, with swollen greyish-brown apices. Internal matrix of stroma moderately developed, 20–50 μm thick, comprising hyaline, gelatinised textura intricata. Subhymenium colorless, 10–15 μm thick, composed of textura angularis. Paraphyses 160–190 × 1.8–2.2 μm, filiform, 0–1-septate, occasionally branched, yellowish-brown in the upper quarter, surrounded by a 0.6–0.8 μm thick gelatinous matrix, gradually irregularly swollen to 3–5 μm and containing disparately-sized oil drops near the apex, forming a 35–50 μm thick epithecium inserted with a number of hyaline, refractive, sub-oblong solid gels 12–32 × 8–14 μm. Asci ripening sequentially, 110–170 × 12–15 μm, cylindrical-clavate, short-stalked, thin-walled, apex bluntly pointed to subtruncate-conical, J–, 8-spored. Ascospores arranged fasciculately, 80–120 × 2–2.4 μm, filiform, hyaline, aseptate, enveloped in a 0.6–0.9 μm thick gelatinous sheath.

Host species, habitat and distribution: Producing ascomata and conidiomata on fallen leaves of *Rhododendron coeloneurum*. Known only from the type locality, Sichuan Province, China.

Comments — *Coccomyces prominens* resembles *C. urceoloides* Spooner in the well-developed excipulum with more or less brown hyphal tips, gradually swollen paraphyses at the apex, and refractive solid gels in the epithecium. However, *C. urceoloides* has smaller, hypophyllous, subepidermal ascomata (350–550 μm diam.) not associated with conidiomata; much smaller, narrowly cylindrical asci (120–135 × 5.5–6 μm); and smaller ascospores (80–98 × ca 1 μm) with a mucilaginous cap at the proximal end (Spooner 1990).

Coccomyces sinensis Y.R. Lin & Z.Z. Li is easily distinguished from *C. prominens* by its triangular to pentagonal ascomata, smaller asci (108–130 × 5.3–6.0 μm), smaller ascospores (54–90 × 1–1.2 μm), subhymenium consisting of textura porrecta-intricata, and distinct paraphyses that are abruptly swollen

to subfusoid-ventricose with a subcylindrical mucro near the apex (Lin et al. 2001).

The similar *C. monticola* Sherwood differs in the smaller and orbicular ascomata (500–800 µm diam.), a thinner covering stroma (20 µm thick), slightly swollen paraphysial apices (2.5 µm wide), and much narrower asci (8–9 µm wide; Sherwood 1980).

Acknowledgements

We are grateful to Dr J.E. Taylor and Dr M. Ye for serving as pre-submission reviewers, and to Dr Y.G. Liu for the field investigations. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31170019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.79

Volume 130, pp. 79–89 *January–March 2015*

Type studies on *Amauroderma* species described by J.D. Zhao et al. and the phylogeny of species in China

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Abstract — Holotypes of eight *Amauroderma* species described by Ji-Ding Zhao and co-workers, together with 93 additional specimens collected from China, were examined. Four names are demoted to synonymy (*A. amoiense* and *A. wuzhishanense* = *A. rugosum; A. dayaoshanense* = *Pyrrhoderma sendaiense; A. fujianense* = *Ganoderma fornicatum*), two represent accepted species (*A. austrosinense*, *A. yunnanense*), and two are of uncertain application (*A. longgangense*, *A. jiangxiense*) because of the poor or sterile condition of the holotype material. Illustrated descriptions of *A. austrosinense* and *A. yunnanense* are given based on the holotypes. Four other species reported from China (*A. perplexum*, *A. rugosum*, *A. subresinosum*, *A. yunnanense*) were sequenced for molecular analysis, and a phylogenetic tree was constructed by maximum parsimony and Bayesian analyses of the nuclear internal transcribed spacer of ribosomal DNA (ITS rDNA) sequences. A key to accepted *Amauroderma* species in China is provided.

Key words — *Ganodermataceae*, morphology, phylogenetics, taxonomy

Introduction

Amauroderma Murrill was established by Murrill and typified by *A. regulicolor* (Berk. ex Cooke) Murrill (Murrill 1905). This genus is mainly characterized by sessile or stipitate (mesopodal, pleuropodal) basidiocarps with a laccate or dull pileus; a trimitic hyphal system; and ellipsoid, subglobose to globose bitunicate basidiospores with an asperulate inner wall (Furtado 1981, Ryvarden 2004). Macroscopically, *Amauroderma* shares with *Ganoderma* P. Karst. a similar basidiocarp shape of central or lateral stipe and a laccate or dull surface. *Ganoderma* can be separated from *Amauroderma* by its distinctly truncate basidiospores, and most *Ganoderma* species grow on dead wood, while most *Amauroderma* species grow in the ground from buried roots (Ryvarden 2004).

Amauroderma is widely distributed in the tropics and subtropics south of 25°N in China within Guangdong, Guangxi, Yunnan, and Hainan Provinces. *Amauroderma* species are regarded as economically valuable because of their famous medicinal properties and pathogenicity (Dai et al. 2007, 2009; Jiao et al. 2013; Chan et al. 2013). Index Fungorum (www.indexfungorum.org) has listed 126 names under this genus. In China, taxonomic research on *Amauroderma* began with Teng, who recorded 10 species (Teng 1936, 1939, 1963). Later Zhao et al. (1979, 1983, 1984) and Zhao & Zhang (1986, 1987a,b, 2000) reported 20 species, nine of which were new. The types of these new species [except *Amauroderma guangxiense* J.D. Zhao & X.Q. Zhang (not traced)] are preserved in the Herbarium Mycologicum Academiae Sinicae (HMAS), the Institute of Microbiology, Chinese Academy of Sciences. Tai (1979) and Fan & Liu (1990) have also contributed to *Amauroderma* records in China.

In this study we investigated the type specimens of *Amauroderma* described by Ji-Ding Zhao and his colleagues and 93 other recently collected specimens from southern China based on morphological characters and rDNA sequence analyses. The taxa are presented below in alphabetical order according to the original names.

Materials and Methods

Morphology

The studied specimens were deposited in the Herbaria of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP); the Institute of Microbiology, Chinese Academy of Sciences (HMAS); and the Institute of Microbiology, Beijing Forestry University (BJFC). Microscopic procedures followed He & Dai (2012). Sections were studied at magnifications up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Measurements were made from sections stained with Cotton Blue. In presenting basidiospore size variation, 5% of measurements are excluded from each end of the range and given in parentheses. The following abbreviations are used: CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, and n = number of basidiospores measured from given number of specimens. Special color terms follow Petersen (1996).

Molecular phylogeny

Amplification of ITS sequences from herbarium specimens was conducted using Phire® Plant Direct PCR Kit (Finnzymes, Finland) with ITS5 and ITS4 primers (White et al. 1990). PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 59 °C for 10 s and 72 °C for 5 s, and a final extension at 72 °C for 2 min. PCR amplification was confirmed on 1% agarose electrophoresis gels

Table 1. ITS sequences of *Amauroderma* and *Tomophagus* species used in the molecular analyses.

Species	Voucher	Locality	GenBank No.
A. perplexum	Cui 6496	Hainan, China	KJ531650
	Dai 10811	Hainan, China	KJ531651
	Wei 5562	Hainan, China	KJ531652
A. rugosum	Cui 6285	Hainan, China	KJ531656
	Zhou 153	Guangxi, China	KJ531657
	Dai 7862	Hainan, China	KJ531658
	Zhou 136	Guangxi, China	KJ531659
	Zhou 347	Guangxi, China	KJ531660
	Zhou 66	Guangxi, China	KJ531661
	Yuan 4554	Hainan, China	KJ531662
	Dai 9904	Hainan, China	KJ531663
	Cui 9011	Guangdong, China	KJ531664
	Cui 9012	Guangdong, China	KJ531665
	Wei 5234	Hainan, China	KJ531666
	Cui 8882	Guangdong, China	KJ531667
	Dai 9553	Hainan, China	KJ531668
	Dai 9566	Hainan, China	KJ531669
	Dai 4345	Hainan, China	KJ531670
	Dai 10040	Hainan, China	KJ531671
	Cui 4078	Fujian, China	KJ531672
	Dai 10307	Hainan, China	KJ531673
	Zhou 523	Guangxi, China	KJ531674
	Zhou 547	Guangxi, China	KJ531675
	Dai 12324	Yunnan, China	KJ531676
	Dai 12390	Yunnan, China	KJ531677
	Cui 9006	Guangdong, China	KJ531678
	THP 30	Unknown	HM 480835
A. subresinosum	Wei 5569	Hainan, China	KJ531649
	ML 288	Malaysia	JQ409358
A. yunnanense	Cui 7974	Yunnan, China	KJ531653
	Dai 13021	Yunnan, China	KJ531654
	Yuan 2253	Yunnan, China	KJ531655
T. colossus	CGMCC 5.763	Philippines	JQ081068

stained with ethidium bromide (Stöger et al. 2006). The PCR products were purified and directly sequenced in Beijing Genomics Institute, China, with the same primers. The most similar sequences (Table 1) were retrieved from GenBank (http://www.ncbi.nlm.gov) using the BLAST option. Sequences were aligned using ClustalX (Thompson et al.

1997). Alignment was manually adjusted to allow maximum alignment and minimize gaps, and deposited in TreeBASE (http://treebase.org/treebase-web/; submission ID: 17050). Identity/similarity between two sequences was calculated using the "pairwise alignment, calculation of the similarity/identity" option of BioEdit v. 7.0.5 (Hall 2005). Maximum parsimony and Bayesian analysis were applied to the ITS dataset. All characters were weighted and gaps were treated as missing data. Maximum parsimony analysis (PAUP* version 4.0b10) was used (Swofford 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to auto-increase, branches of zero length were collapsed, and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all trees generated under different optimality criteria.

JModelTest (Posada 2008) was used to determine the best-fit evolution model for each data set for Bayesian inference (BY). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four simultaneous Markov chains were run with 3 million generations, and trees were sampled every 100 generations. Trees generated for the first one-fourth generations were discarded as burn-in. A 50% majority rule consensus tree of all remaining trees was calculated. Branches that received maximum parsimony bootstrap support (BSS) and Bayesian posterior probabilities (BPP) respectively greater than or equal to 75% and 0.95 were considered as significantly supported.

Taxonomy

We studied eight species of *Amauroderma* (*Ganodermataceae*) described from China by Ji-Ding Zhao et al. Four of the eight names are synonymized, two are accepted, and two are of uncertain application.

Amauroderma amoiense J.D. Zhao & L.W. Hsu, Acta Mycol. Sinica 2: 164. 1983.

Holotype: China. Fujian Prov., Xiamen, sandy land, 13.VI.1976, Li Hui-zhong & Zhang Xiao-qing 28 (HMAS 42784).

= ***Amauroderma rugosum*** (Blume & T. Nees) Torrend, Brotéria, Sér. Bot. 18: 127. 1920.

The type material is sterile, but all other characters fit *A. rugosum* well. For a detailed description of *A. rugosum*, see Ryvarden & Johansen (1980).

Amauroderma austrosinense J.D. Zhao & L.W. Hsu, Acta Mycol. Sinica 3: 20. 1984. Fig 1

Holotype: China. Hainan Prov., Bawangling, ground, 20.IV.1977, Han Shu-jin 902 (HMAS 42695).

Basidiocarps annual, stipitate, umbelliform. Pileus single, suborbicular to circular, glabrous, dull, clay-buff to fulvous brown, densely concentrically zoned

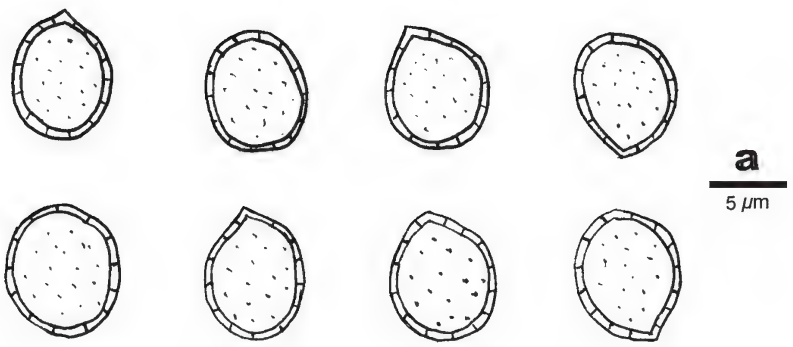

FIG. 1. *Amauroderma austrosinense* (holotype): basidiospores.

and slightly irregularly radially furrowed; MARGIN obtuse and deflexed when dry; STIPE sub-central or eccentric, concolorous with pileus, cavitate, up to 12 cm long and 1 cm thick. CONTEXT cinnamon, dense and hard, homogenous, up to 4 mm thick. PORE SURFACE cream white when fresh, turning brown with age; PORES circular, entire, 6–8 per mm. TUBES woody, up to 3 mm long.

HYPHAL SYSTEM trimitic; GENERATIVE HYPHAE with clamp connections, hyaline, thin-walled, 2.8–4 µm in diam; SKELETAL HYPHAE pale yellow to almost colorless, sparsely branched, 3.7–5.2 µm in diam; BINDING HYPHAE hyaline, branched, tortuous, 1.5–2.2 µm in diam; all the hyphae IKI–, CB+; tissues darkening in KOH. BASIDIOSPORES subglobose to globose, faintly yellowish, IKI–, CB+, doubled-walled, exospore smooth, endospore with conspicuous echinule, (6.5–)6.8–7.6(–8) × (5.9–)6.2–7(–7.3) µm, L = 7.34 µm, W = 6.58 µm, Q = 1.12 (n = 30/1).

ADDITIONAL SPECIMEN EXAMINED: **CHINA, HAINAN PROV.**, Changjiang County, Bawangling Nat. Res., ground, 20.IV.1977 (HMAS 42695).

REMARKS: *Amauroderma austrosinense* is an accepted species, remarkable for its umbelliform basidiocarps with distinct concentric rings, small pores, and basidiospores. *A. camerarium* (Berk.) J.S. Furtado is similar to *A. austrosinense* by sharing a clay-colored, densely concentrically zoned, and radially furrowed pileus. However, *A. camerarium* differs in its larger basidiospores (12–15 × 10–13 µm; Ryvarden 2004).

Amauroderma leucosporum Corner may be confused with *A. austrosinense* by having similar-sized subglobose to globose basidiospores and also not turning blood red on bruising, but *A. leucosporum* can be distinguished by its finely to distinctly villous pileus and stipe (Corner 1983).

Amauroderma dayaoshanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 5. 1987.

Holotype: China. Guangxi Auto. Reg., Dayaoshan, dead wood, 1982, Wei Bing-gang 70 (HMAS 48282).

= ***Pyrrhoderma sendaiense*** (Yasuda) Imazeki, Trans. Mycol. Soc. Japan 7: 4. 1966.

The type is sterile but has the characteristics of *P. sendaiense* (see Núñez & Ryvarden (2000) for a detailed description).

Amauroderma fujianense J.D. Zhao, L.W. Hsu & X.Q. Zhang, Acta Microbiol. Sinica 19: 275. 1979.

Holotype: China. Fujian Prov., Sanming County, rotten wood, 28.VI.1976, Zhang Xiao-qing 68 (HMAS 37919).

= ***Ganoderma fornicatum*** (Fr.) Pat., Bull. Soc. Mycol. Fr. 5: 71. 1889.

The holotype is sterile but represents a juvenile specimen of *G. fornicatum* (see Patouillard (1889) for a detailed description).

Amauroderma jiangxiense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 206. 1987.

Holotype: China. Jiangxi Prov., Ji'an County, rotten wood, 15.III.1983, Yang Ren-gen 152 (HMAS 50418).

The basidiocarp is umbelliform and the pileus is black, laccate, and shiny, but the specimen is completely sterile. It probably represents a *Ganoderma* species, but the application of the name is uncertain.

Amauroderma longgangense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 5: 222. 1986.

Holotype: China. Guangxi Auto. Reg., Nonggang, on rotten wood, 1979, Wei Bing-gang 790111-G8 (HMAS 47621).

Basidiospores subglobose, 6–8.5 × 5–8 µm, with spinules 0.8–1.2 µm on sporoderm. However, the single-walled basidiospore obviously differs from the double-walled spore of other *Amauroderma* species. The application of the name is uncertain.

Amauroderma wuzhishanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 208. 1987.

Holotype: China. Hainan Prov., Wuzhishan, on rotten wood, 22.VII.1956, Jiang Guang-zheng (HMAS 19311).

= ***Amauroderma rugosum*** (Blume & T. Nees) Torrend, Brotéria, Sér. Bot. 18: 127. 1920.

The holotype is in accordance with *Amauroderma rugosum*, with its pileus obscurely zonate, radially quite rugose, and warted. For a detailed description of *A. rugosum*, see Ryvarden & Johansen (1980).

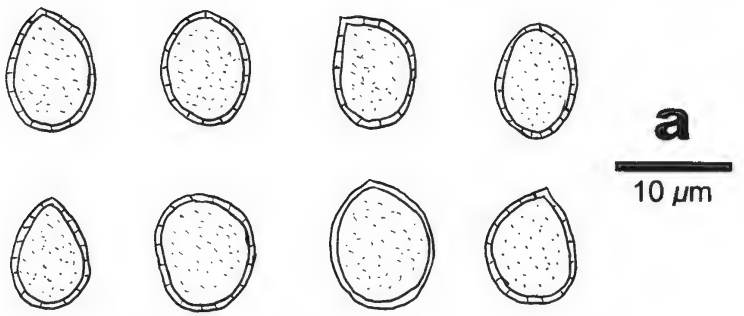

Fig. 2. *Amauroderma yunnanense* (holotype): basidiospores.

Amauroderma yunnanense J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica, Suppl. 1: 268. 1987 ["1986"]. Fig 2

Holotype: China. Yunnan Prov., Xichou County, ground, Xiaoqiaogou, 14.V.1959, Wang Qing-zhi 89 (HMAS 48231).

Basidiocarps annual, centrally to laterally stipitate, coriaceous to corky, brittle when dry. Pileus single, flabelliform, suborbicular, umbilicate, Pileal surface pale yellowish brown to dark brown, adpressed velutinate, with distinct concentric zones at the margin and fine furrows in the middle; margin inflexed, acute or subacute, entire or slightly lacerated and thin; stipe concolorous with the pileus, up to 7 cm long, inflated at base. Context homogeneous, white to pale yellow. Pore surface white when fresh, pale straw yellow when dry, bruising unchanged; pores angular, 2–3 per mm; Tubes concolorous with pore surface, up to 5 mm thick.

Hyphal system trimitic; generative hyphae with clamps, hyaline, thin-walled, 2–4.3 μm in diam; skeletal hyphae thick-walled, pale yellow, arboriform, 3.5–5.5 μm in diam; binding hyphae hyaline, branched, tortuous, 1.8–2.5 μm in diam; all the hyphae IKI–, CB+; tissues darkening in KOH. Basidiospores broadly ellipsoid to subglobose, pale yellow brown, IKI–, CB+, doubled-walled, exospore smooth, hyaline, endospore with conspicuous echinule, (8.3–)8.9–10.3(–10.7) × (6.3–)6.7–8(–8.8) μm, L = 9.35 μm, W = 7.32 μm, Q = 1.22–1.34 (n = 120/4).

Additional specimens examined: **China, Yunnan Prov.**, Chuxiong, Zixishan Forest Park, ground, 8.IX.2006, Yuan 2253 (IFP 013207); Ailaoshan, 5.IX.2007, Yuan 3367 (IFP 013182); Kunming, Qiongzhusi Park, 21.X.2009, Cui 7974 (BJFC 006463); Xiaoshao Village, 22.IX.2012, Dai 13021 (IFP 019123).

Remarks: *Amauroderma yunnanense* is an accepted species characterized by a brown tomentose pileus and stipe.

Amauroderma perplexum Corner and *A. yunnanense* both have a velutinate pileus and stipe as well as stipes that dilate at the base; *A. perplexum* can be

separated by larger basidiospores, longer tubes, and a pore surface that changes color when bruised (Corner 1983).

Amauroderma partitum (Berk.) Wakef., which may be confused with *A. yunnanense* as they share similar angular pores that are 2–3 per mm, can be distinguished by its glabrous, slightly shiny pileus and larger basidiospores (12–15 × 8–10 µm; Ryvarden 2004).

Molecular phylogeny

The nuclear ITS rDNA dataset contains 32 sequences representing *A. perplexum*, *A. rugosum*, *A. subresinosum* (Murrill) Corner, and *A. yunnanense*, with *Tomophagus colossus* (Fr.) Murrill as outgroup (Table 1). After the ambiguous sites at both ends and the 5.8S region were trimmed off, the alignment comprised 561 characters, of which 462 were constant, 22 were variable but parsimony-uninformative, and 77 were parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (CI = 0.918, RI = 0.952, RC = 0.874, HI = 0.082). A strict consensus tree of these trees is presented in Figure 3. The 50% majority consensus tree generated by the Bayesian analysis showed a similar topology with the strict consensus MP tree, and only the topology from MP analysis is presented, while both bootstrap support value (BSS) and Bayesian posterior probabilities (BPP) are shown at the nodes (Fig. 3).

In the phylogenetic tree, each sampled *Amauroderma* species was resolved as a strongly supported clade (100% BSS and 1.00 BPP). Identity/similarity calculation result shows that identities of *Amauroderma* sequences are between 0.98 and 1. We failed to obtain sequences from the type specimens of *Amauroderma* described by Ji-Ding Zhao from China as the materials were too old to extract integrated DNA.

Key to species of *Amauroderma* in China

1. Pileal surface appressed velutinate 2
1. Pileal surface glabrous 3

2. Pores 2–3 per mm, pore surface straw yellow, not reddening on bruising, spores (8.3–)8.9–10.3(–10.7) × (6.3–)6.7–8(–8.8) µm..... ***A. yunnanense***
2. Pores 4–6 per mm, pore surface cream white, reddening on bruising, spores (10.3–)10.8–14(–14.8) × (8.9–)9.2–12(–13.1) µm..... ***A. perplexum***

3. Pileus surface laccate, spores ovoid to broadly ellipsoid, (13.7–)14.6–17.1(–17.5) × (8.3–)8.9–10.2(–11.3) µm..... ***A. subresinosum***
3. Pileal surface dull 4

4. Pileal clay-buff to fulvous brown, pores 7–8 per mm, spores (6.5–)6.8–7.6(–8) × (5.9–)6.2–7(–7.3) µm ..... ***A. austrosinense***
4. Pileus taupe, dark brown to black, pores 4–6 per mm, spores (8.1–)8.4–11.7 (–12) × (7–)7.5–9.6(–10.3) µm..... ***A. rugosum***

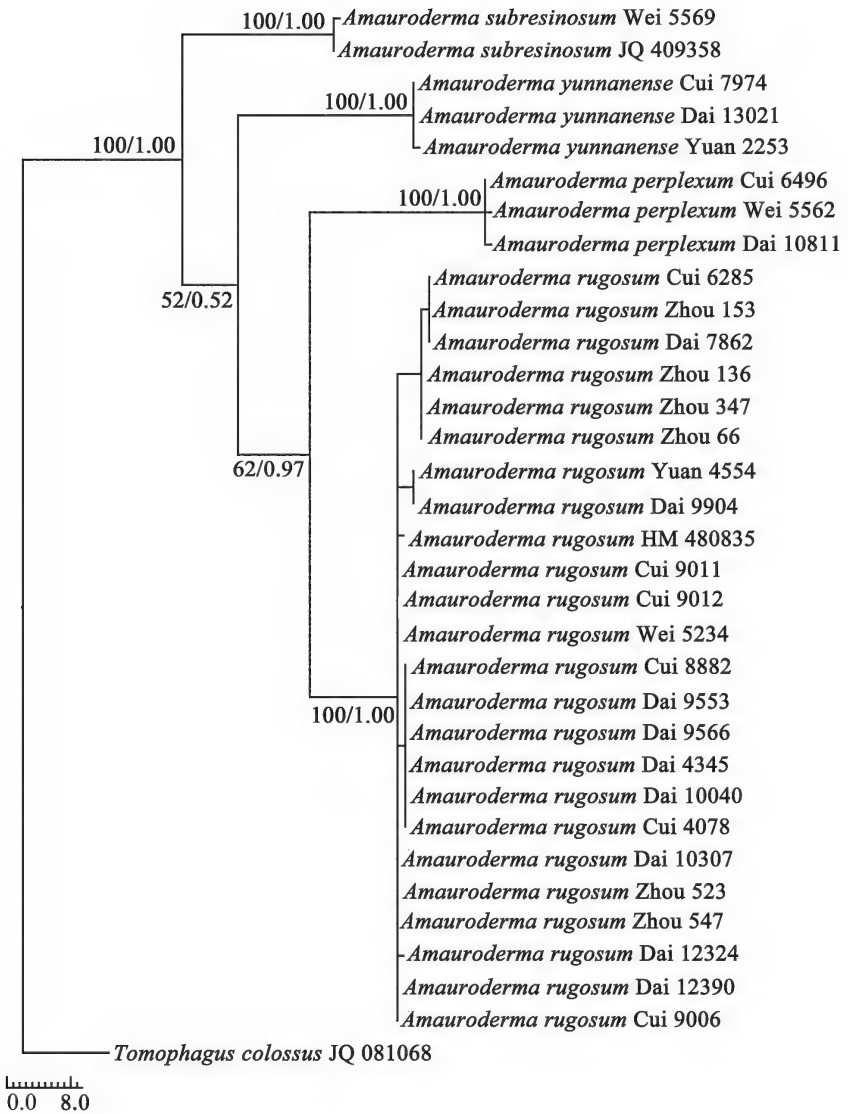

FIG. 3. Strict consensus tree obtained from Maximum Parsimony and Bayesian analyses of ITS sequences of *Amauroderma*. *Tomophagus colossus* was used as outgroup. Numbers at branches indicate parsimony bootstrap values and Bayesian posterior probabilities values higher than 50%.

Acknowledgements

The authors are grateful for presubmission review and helpful comments by Bao-Kai Cui (Institute of Microbiology, Beijing Forestry University) and Xiao-Yong Liu (Institute of Microbiology, Chinese Academy of Sciences). Special thanks are due to Dr. Katherine F. LoBuglio (Harvard University Herbaria, USA) for checking and improving the manuscript. This research was financed by the National Natural Science Foundation of China (Project Nos. 31170022 & 31470148).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.91

Volume 130, pp. 91–103 *January–March 2015*

Cladonia corymbescens consists of two species

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Abstract — The lichen *Cladonia corymbescens* (*Cladoniaceae*, *Ascomycota*) has been reported to have two main ranges, one in Melanesia and Australasia and another in the Himalayas and surrounding areas. When specimens from New Caledonia, Bhutan, and Thailand were subjected to molecular sequencing (ITS rDNA and RPB2), two distinct clades were detected. The clades were also supported by morphological and geographical differences supporting recognition of two distinct species. *Cladonia corymbescens* s. str. is present in the Philippines, Melanesia, and Australasia, while the South East Asian mainland material is referred to *C. pseudofissa,* a new combination at species level.

Key words — *Cladonia furcata* group, lichens, molecular systematic, phylogeny, taxonomy

Introduction

One of the most common fruticose species of *Cladonia* in the mountains of western Melanesia is *Cladonia corymbescens*. The nomenclature of this species and its similarities with other species was thoroughly discussed by Stenroos (1988), who also reported it from China and Nepal. However, Abbayes (1974) was the first lichenologist to recognize this species from the Himalayas. His opinion was also accepted by Awasthi & Ahti (2007) and Rai et al. (2014), who stated that the species is widespread in the mountains in India and Nepal. In his world monograph, Vainio (1887) could not give a definite opinion on the type material of *C. corymbescens* from New Caledonia, and he referred the only Himalayan specimen representing that species that he apparently examined to *C. erythrosperma* var. *thomsonii*.

Several studies using molecular data have shown that many morphological *Cladonia* species are not monophyletic (Kotelko & Piercey-Normore 2010, Fontaine et al. 2011, Pino-Bodas et al. 2011, Steinová et al. 2013). The re-examination of the morphology based on molecular data in lichenized fungi has revealed that in several cases some phenotypical differences have been overlooked (Argüello et al. 2007, Núñez-Zapata et al. 2010, Parnmen et al.

2013, Muggia et al. 2014). The aim of this study was to use DNA sequence data to assess whether *Cladonia corymbescens* is a genetically homogeneous species and to revise its classification based on the phylogenetic results and morphological differences.

Materials & methods

Material for DNA analyses

Altogether 39 specimens of 29 species of *Cladonia* representing the supergroup *Cladonia* in the provisional classification of Stenroos et al. (2002) were selected for a phylogenetic analysis in this study. These specimens are preserved in the herbaria of University of Helsinki (H), University of Turku (TUR) or Complutense University, Biology Faculty (MACB); details of the collections are shown in Table 1. Four specimens of *Cladonia corymbescens*, two from New Caledonia and two from the Himalayas, were available for DNA analyses, while 60 sequences had been generated in previous studies (Stenroos et al. 2002; Pino-Bodas et al. 2010a, 2010b, 2012a, 2012b, 2012c, 2013a, 2013b). *Cladonia rangiformis* Hoffm. was selected as outgroup based on previous results (Stenroos et al. 2002).

DNA extraction, PCR, and sequencing

Total DNA was extracted using DNeasy Plant Mini Kit (QUIAGEN, Germany) following the manufacturer's instructions. In this study two genetic regions were chosen: internal transcribed spacer of ribosomal DNA (ITS rDNA) and the second largest subunit of RNA polymerase II (RPB2). Although the ITS rDNA region has been chosen as standard barcoding for fungi (Schoch et al. 2012), in *Cladonia* the ITS rDNA frequently fails in the identifications (Kelly et al. 2011; Pino-Bodas et al. 2013b) and RPB2 gives better results (Pino-Bodas et al. 2013b). The primers used to amplify the nuclear ITS nrDNA were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and for RPB2 they were RPB2dRaq/RPB2rRaq (Pino-Bodas et al. 2010a) or CLRPB25F/CLRPB27R (Yahr et al. 2006). The amplification programs are listed in Pino-Bodas et al. (2013a). PCR was carried out with Ready-to-Go-PCR Beads (GE Healthcare Life Sciences, UK). Amplifications were prepared for a 25 µL final volume. PCR was performed using a Mastercycler ep Gradient S (Eppendorf, Westbury, NY). PCR products were purified with ExoSAP-IT (USB Corporation, USA) and sequenced in Macrogen Europe (Amsterdam, Netherlands).

Alignments and phylogenetic analyses

The forward and reverse strands were assembled and edited in Sequencher™ v. 4.1.4 (Gene Codes Corporation, Inc., Ann Arbor, Michigan, USA). BLAST searches were used to confirm that the obtained sequences belonged to *Cladonia*. The sequences were manually aligned in BIOEDIT (Hall 1999). Nine ambiguous positions were manually delimited and removed from the ITS rDNA alignment. The RPB2 alignment did not have ambiguous positions.

Every region was separately analyzed by Maximum Likelihood (ML), with 500 bootstrap replicates to assess the support of each node. The conflict between the loci was tested according Lutzoni et al. (2004). This method considers the existence of

TABLE 1. List of *Cladonia* specimens used in the molecular study with voucher specimen information and GenBank accession numbers.
(The new sequences and the new combination are in bold font.)

SPECIES	COLLECTION	CODE	ITS rDNA	RPB2
C. acuminata	USA, Ahti 63278 (H)	1ACUMI	JN621932	JN621965
C. apodocarpa	USA, Harris 54250 (H)	1APODO	KC526127	KC526068
C. borbonica	New Caledonia, Dennetière 109 (H)	AT686	AF455214	**KP732364**
C. cariosa	Spain, Burgaz (MACB 94208)	5CARI	JN621909	JN621941
C. cartilaginea	Brazil, Stenroos 4926 (TUR)	LK45	AF455212	—
C. ceratophylla	Brazil, Stenroos 5081 (TUR)	LK37	AF455171	—
C. conista	Spain, Burgaz (MACB 92796)	2HUMIL	JF926613	JF926567
	Russia, Zolotuchin 26a (H)	1CONIST	JF926633	JF926568
C. corsicana	Spain, Burgaz (MACB 100763)	SP1	JF288797	JF288833
	Spain, Burgaz (MACB 101074)	SP2	JF288798	JF288834
C. corymbescens	New Caledonia, Dennetière 45 (TUR)	AT680	AF455235	**KP732366**
	New Caledonia, Christenhusz 6169d (H)	CL166	**KP732363**	**KP732367**
C. cyathomorpha	Spain, Burgaz (MACB)	1CYATH	KC415941	KC525275
	Norway, Ahti 68660 & Tønsberg (H)	3CYATH	KC415943	KC525276
C. firma	Spain, Burgaz (MACB 91619)	1FIRM	FM205907	FM207568
	Spain, Burgaz (MACB 90655)	7FIRM	FM205910	FM207576
C. foliacea	Portugal, Burgaz (MACB 90506)	1FOL	FM205894	FM207569
C. furcata	USA, Ahti 58283 (TUR)	AT638	AF455220	**KP732369**
C. humilis	Spain, Burgaz (MACB 95931)	9HUMIL	JF926615	JF926576
	Croatia, Burgaz (MACB 101103)	20HUMIL	JF926621	JF926580
C. multiformis	Canada, Ahti 57065 (H)	LK70	AF455213	**KP732370**
C. nana	Brazil, Stenroos 4940 (TUR)	LK34	AF455211	—
C. neozelandica	New Zealand, Wirth 28180 (TUR)	AT590	AF455206	—
C. petrophila	USA, Ahti 56654 (H)	LK68	AF455222	—
C. peziziformis	USA, Stenroos 5198 (TUR)	AT631	AF455221	—
	USA, Ahti 56670 (H)	LK38	AF455182	—
C. pityrophylla	Brazil, Stenroos 5096 (TUR)	LK40	AF455238	—
C. pseudofissa	Thailand, Parnmen 285 (H)	1CORYMB	**KP732362**	**KP732365**
	Bhutan, Søchting 9206 (H)	LK35	AF455239	**KP732368**
C. pulvinata	Spain, Burgaz (MACB 91646)	4PUL	FM205911	FM207579
C. pulvinella	USA, Ahti 69191 (H)	CLCAL4	KC415963	KC525287
C. pyxidata	Greenland, Hansen (H)	17PYXI	KC415983	KC525269
C. rangiformis	Netherlands, Van der Goes et al. (H)	5RANG	JN811400	JN811429
	Sweden, Skytén (H)	6RANG	JN811401	JN811430
C. scabriuscula	Canada, Ahti 56969 (H)	LK11	AF455217	**KP732371**
C. subcariosa	USA, Moore (H)	1SUBCARI	JN621936	JN621969
C. subconistea	Korea, Moon 7188 (H)	1SUBCONI	KC415949	KC525296
C. subulata	Spain, Burgaz (MACB 93151)	1SUBU	FN86566	HM243210
C. turgida	Canada, Lendemer (H)	1TURG	JF288801	KC526089

incongruity whenever a clade was supported with a bootstrap of more than 75% in a locus, while in other loci the individual sequences of this clade were part of another clade with bootstrap support ≥75%. The trees were checked manually. No conflicts were detected and the datasets were combined. The combined dataset was analyzed by Maximum Parsimony (MP), ML, and Bayesian analyses. MP analysis employed PAUP version 4.0.b.10 (Swofford 2002), using heuristic searches with 1000 random taxon-addition replicates with TBR branch swapping and MulTrees option in effect, and equally weighted characters and gaps treated as missing data. Bootstrap with 1000 replicates was used as the confidence analysis, using the heuristic option. The ML and Bayesian analyses were run considering four partitions (ITS rDNA and each codon position of RPB2). Maximum Likelihood analyses (ML) were done in RAxML 7.04 (Stamatakis 2006), assuming the model GTRGAMMA. A fast bootstrap with 500 replicates was implemented to assess the support of each node. Bayesian analysis was carried out using the program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The models of evolution were selected with MrModeltest (Nylander 2004) under AIC criterion. The models selected were GTR+I+G for ITS rDNA and SYM+I+G for RPB2. Posterior probabilities (pp), approximated by sampling trees using Markov Chain Monte Carlo (MCMC), of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. Model parameters were estimated in each analysis for 5,000,000 generations sampled in four simultaneous chains, and every 1000th was saved into a file. When the analysis was finished we checked that standard deviation between the runs was <0.005. The program AWTY (Nylander et al. 2008) was used to determine when the chains reached the stationary stage. The first 1000 trees were deleted as the "burn in" and the 50% majority-rule consensus tree was calculated using the "sumt" command of MrBayes.

Morphology and chemistry

About 63 herbarium specimens of *Cladonia corymbescens* s. lat. deposited in Helsinki (H) were morphologically re-evaluated after the phylogenetic analyses. Authors Ahti and Stenroos had previously studied many additional specimens in various herbaria [e.g., Geneva (G), Leiden (L), Lucknow (LWG, LWG-LWU, LWG-AWAS)] as well as material borrowed from Edinburgh (E); see also the specimen lists in Stenroos (1988) and Rai et al. (2014).

The secondary metabolites were analyzed by thin layer chromatography (TLC) according to standardized procedures (Orange et al. 2001) using the solvents A (Toluene: dioxane: acetic acid, Merck) and B (Hexane: diethylether: formic acid, Merck).

Results

In this study we generated 10 new sequences (2 of ITS rDNA and 8 of RPB2). The combined dataset contained 1332 unambiguous characters (597 of ITS rDNA and 735 of RPB2), 267 of which were parsimony informative (152 of ITS rDNA and 115 of RPB2). The MP analysis generated two equally parsimonious trees of 886 steps. The ML analyses of the combined dataset generated a tree with likelihood value of –LnL = 6355.82, while the mean of likelihood of the Bayesian analysis was –LnL = 6589.51.

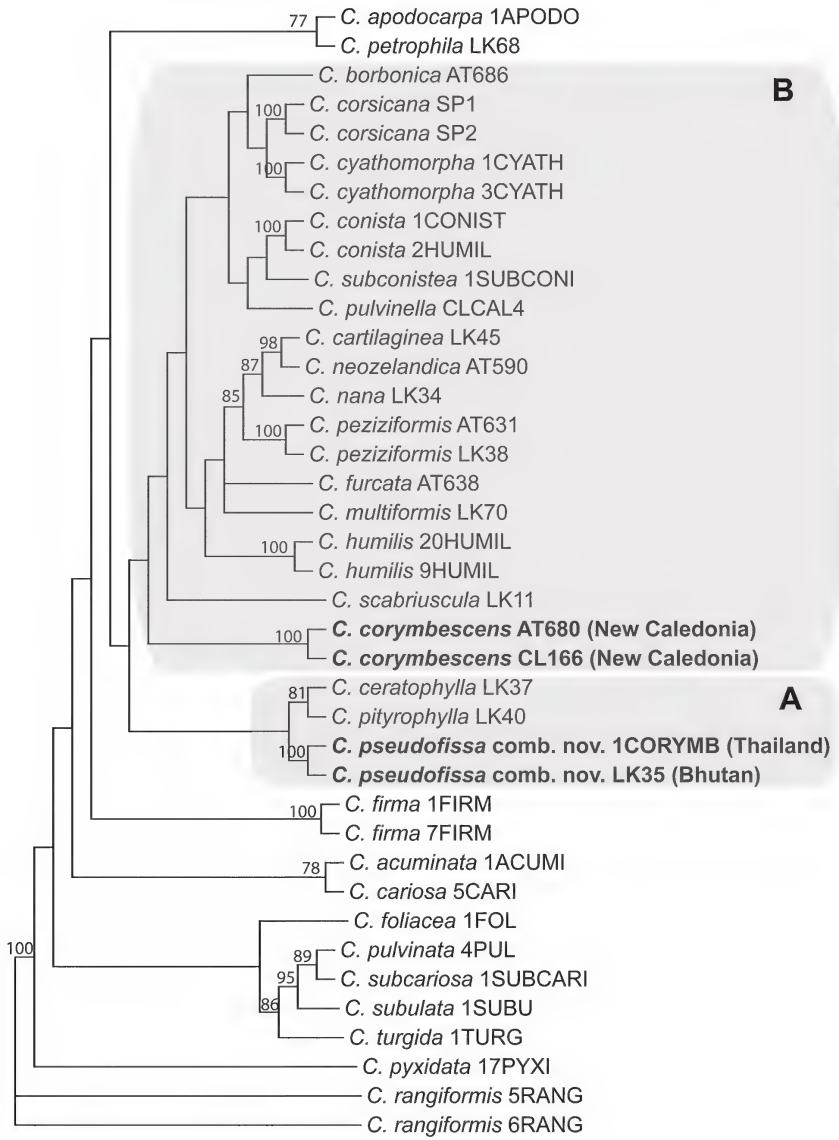

FIG. 1. *Cladonia* spp. phylogeny: consensus tree of the two most parsimonious trees based on the concatenated matrix of ITS rDNA and RPB2. The numbers on the branches represent bootstrap values (>75%).

In all the analyses the specimens from the Himalayan region and the specimens from New Caledonia formed two clades; however, the relationships

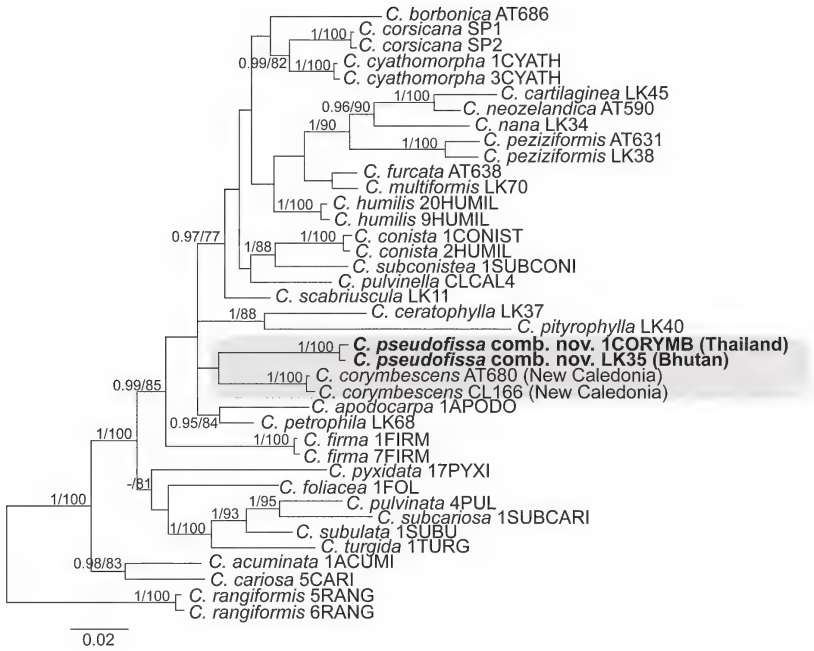

FIG. 2. *Cladonia* spp. phylogeny: 50% consensus majority tree of Bayesian analysis based on the concatenated matrix of ITS rDNA and RPB2. Posterior probability of bayesian analysis (>0.95) and bootstrap values of ML analyses (>75%) are shown on the branches.

were not the same in all the analyses. In the MP analysis (FIG. 1) the specimens from the Himalayan region (in the tree *C. pseudofissa*) are closely related with *C. pityrophylla* Nyl. and *C. ceratophylla* (Sw.) Spreng. (FIG. 1, clade A), but this relationship was not well supported (bootstrap <70%). The specimens from New Caledonia cluster in a clade with specimens representing the *C. furcata* group, *C. humilis* group, *C. corsicana* (Rondon & Vězda) Pino-Bodas et al., *C. borbonica* Nyl., *C. cartilaginea* Müll. Arg., and *C. neozelandica* Vain. (FIG. 1, clade B). The ML and Bayesian analyses generated trees with the same topology, and only the 50% consensus majority-rule tree from Bayesian analyses is shown (FIG. 2). The specimens from New Caledonia (*C. corymbescens*) and the specimens from the Himalayan region (*C. pseudofissa*) formed two clades that are closely related, although the relationship was not supported in any of the analyses.

Other differences between the MP tree and the Bayesian tree are that *C. pyxidata* (L.) Hoffm. was shown as basal in the MP analysis, while the Bayesian analysis showed *C. cariosa* (Ach.) Spreng. and *C. acuminata* (Ach.) Norrl. in the basal clade. Additionally, the clade of *C. conista* (Nyl.) Robbins,

C. subconistea Asahina, and *C. pulvinella* S. Hammer is closely related to a clade of *C. cyathomorpha* Stirt. ex Walt. Watson, *C. corsicana*, and *C. borbonica* in the MP analysis, but not in the Bayesian analysis.

Taxonomy

Cladonia corymbescens Nyl. ex Leight., Ann. Mag. Nat. Hist., ser. 3, 18: 407. 1866. FIG. 3A

TYPE (Abbayes 1974: 112): New Caledonia, Mont de M'bée, 1855–1860 E. Vieillard 1785 (PC, lectotype, G, H-NYL 38414, PC-Lenormand, isolectotypes). [The lectotypification by Abbayes was overlooked by Ahti in Stenroos (1988: 126), Ahti (1993) and other recent authors, but the type collection cited is the same].

= *Cladonia novoguineensis* Zahlbr., Bot. Jahrb. Syst. 62: 455. 1929.

TYPE: Papua New Guinea ["Nordöstliches Neu-Guinea, Kaiser Wilhelmsland"], Morobe Prov., Sarawaged Mts. ["Saruwaged-Gebirg"], 3600–4000 m, C. Keysser 66 ["68"] (W, holotype).

Primary thallus 0.5–1 × 1.5–2 mm, soon disappearing. Podetia (2–)3–7 cm tall, 0.7–1.5 mm thick, pale to dark brown, medulla hardly melanotic at base, erect, dichotomously, trichotomously or irregularly richly branched, axils open, at least some podetia stout, sterile podetia have thin, subulate tips, fertile podetia becoming thicker, forming characteristic lateral fissures and perforations, branches often flattened. Surface highly discontinuously corticated, especially towards the base, in part sparsely microsquamulose or with larger squamules, sometimes verruculose, apical parts hardly pruinose. Podetial wall 120–220 µm thick, with indistinct cortex 10–20 µm, medulla 150–200 µm, and stereome 50–60 µm, hard. Pycnidia terminal or sometimes lateral on podetia, rarely on basal squamules, containing hyaline slime, 1.5 mm in diam, conidia 6–7 × 1 µm. Apothecia 0.8–1 mm wide, terminal, on every tip on fertile podetia, sometimes deformed, brown, spores not observed.

CHEMISTRY—K+ yellow, PD+ red, rarely PD–. CHEMOTYPE 1: Atranorin (major), fumarprotocetraric acid (major), protocetraric acid (minor), confumarprotocetraric acid (minor), rarely rangiformic and norrangiformic acids, occasionally 1–4 unknown minor substances and 1–3 terpenoids. CHEMOTYPE 2: Atranorin only, the fumarprotocetraric acid complex lacking (then PD–) (Stenroos 1988). If chemotype 2 proves to represent a distinct taxon, the name *Cladonia novoguineensis* is applicable for that (no fresh material for DNA analysis was available).

HABITAT—Terrestrial in bogs, grasslands, road banks, rarely on tree stumps, at 1500–4400 m in Papua New Guinea (Stenroos 1988: 126).

DISTRIBUTION—Australia (New South Wales, Queensland), Indonesia (Java), New Caledonia, New Zealand (North Island), Papua New Guinea, Philippines (Luzon).

Additional specimens examined (See also Stenroos 1988) — **NEW CALEDONIA**, between Yaté and Goro, 1966, Hill 11741 (BM, H). **PAPUA NEW GUINEA**, **Eastern Highlands**, Mt. Wilhelm, Pindaunde Lake, 3540 m, 1965, A. C. Jermy 5327 (BM, H; richly fertile!); **Morobe Prov.**, Mt. Sarawaket Southern Range, 4 km NEE of Lake Gwam, 2850 m, 1981, T. Koponen 31662 (H, LAE); **Southern Highlands**, Mt. Giluwe, 3500 m, 1982, H. Streimann 24151 (CANB, H, LAE; chemotype 2). **PHILIPPINES**, **Luzon**, Mountain Prov., Alab on road Baguio to Bontoc, 850 m, 1976, W. S. Gruezo 2030 (CAHP, H).

Cladonia pseudofissa (Asahina) Ahti, Pino-Bodas & S. Stenroos, **stat. et comb. nov.** Fig. 3B

MycoBank MB 809612

≡ *Cladonia rangiformis* var. *pseudofissa* Asahina, Fl. E. Himalaya: 595. 1966.

Type: India, Sikkim, Jongri, 4000 m, 1960, M. Togashi 157 (TNS, holotype).

= *Cladonia erythrosperma* var. *thomsonii* Vain., Acta Soc. Fauna Fl. Fenn. 4: 376. 1887, syn. nov. [non *Cladonia thomsonii* Ahti].

Type: India, "India orientalis", [1847–51], J. D. Hooker & T. Thomson 2145 (PC, holotype).

= *Cladonia rangiformis* var. *incurva* Müll. Arg., Flora 74: 72. 1891, syn. nov. [non *Cladonia incurva* Ahti].

Type: India, Himachal Pradesh (?), Damodar ["Damdar"] Valley, 4200 m, J. F. Duthie (BM, holotype).

The earliest names for *C. pseudofissa* are *C. erythrosperma* var. *thomsonii* and *C. rangiformis* var. *incurva*, but they cannot be adopted at species level because these epithets are already occupied in *Cladonia* by *C. thomsonii* Ahti (1978) and *C. incurva* Ahti (1961).

Primary thallus rarely visible, soon disappearing, consisting of minute squamules. Podetia 1–4(–5) cm tall, to 1 mm thick, almost white, ashy grey or more rarely dark brown, medulla clearly melanotic at base; often very delicate, ascendant to erect, usually somewhat curly in appearance, without any stouter sterile podetia, repeatedly subcorymbosely dichotomously branched, apices subulate, ascyphose, axils usually perforated. Fertile podetia somewhat thicker. Surface very matt, minutely fibrous or pruinose, often very smooth and almost continuously corticate, with a few very small squamules, longitudinally somewhat fissured, esorediate. Podetial wall 150–160 µm thick, with distinct cortex 10–15 µm, medulla 75–100, stereome 25–50 µm thick, softish rather than cartilaginous. Pycnidia always at tips of podetia, shortly stalked, pyriform, black, c. 2 × 1 mm; conidia or colour of slime not observed. Apothecia dark brown, 0.5–1 mm, subglobose, on tips of flattened branchlets, spores not observed.

Chemistry—K+ yellow, PD+ orange red. Contains atranorin and the fumarprotocetraric acid complex.

Habitat—On ground, often on thin soil over rocks.

FIG. 3. A: ***Cladonia corymbescens*** (Papua New Guinea, T. Koponen 31662, H).
B: ***Cladonia pseudofissa*** (Nepal, P. Ozenda 147, H). Scale bar = 1 cm.

DISTRIBUTION—Bhutan, China (Sichuan, Tibet, Yunnan), India (Arunachal Pradesh, Himachal Pradesh, Kerala, Sikkim, Tamil Nadu, Uttarkhand, West Bengal), Malaysia (Sabah), Nepal, Thailand.

ADDITIONAL SPECIMENS EXAMINED — **BHUTAN**, **PARO DISTRICT**: below Jaley la Dzong, 2500-3000 m, 1998, U. Søchting 9206 (C, H; DNA voucher, Stenroos et al. (2002)

and GenBank, as *C. corymbescens*). **CHINA**, **Sichuan**: Miyi Co., 3200 m, 1983, L. S. Wang 83-834 (H, KUN). **Tibet**: Kang Ding Co., 1976, Z. Mu 5304 (H, KUN); **Yunnan**: Lijiang Co., 1985, L. S. Wang 85-140 (H, KUN). **INDIA**, **Sikkim**: North Sikkim District, Phune–Yakche, 3000 m, 1996, G. P. Sinha 1103 (ASSAM, H). **Uttarakhand**: Pithoragarh District, Satgarh–Dhawj, 2700 m, 1989, D. K. Upreti L-18438 (H, LWG). [For details of further Indian collections, see Rai et al. 2014, as *C. corymbescens.*] **NEPAL**, **Farwestern Region**: Seti, Dadachaur, Nhuna Khola, 2700 m, 1973, P. Ozenda 147C (G, H).

Discussion

Cladonia pseudofissa was included by Abbayes (1974) in *C. corymbescens.* He also thought that *C. perfossa* Nuno, described from Taiwan, belongs here. However, as discussed by Ahti & Lai (1979) and Stenroos (1988: 126), *C. perfossa* is probably a distinct species (podetia short and thick; a topotype re-examined in H), although still very poorly known.

Asahina (1966) clearly included the present species in *C. rangiformis* because he found that it contains atranorin in addition to fumarprotocetraric acid; such a strain is known in *C. rangiformis*. In habit *C. pseudofissa* does resemble *C. rangiformis* to some extent, but *C. rangiformis* is not known to range east of northwestern Iran (Litterski & Ahti 2004: 233). They are distinguished by the longitudinally split podetia and melanotic part at the base of *C. pseudofissa*.

The two segregated species generally differ in habit and colour, *C. corymbescens* being taller, erect, brownish (much resembling *C. furcata* (Huds.) Schrad.) while *C. pseudofissa* is smaller, thinner, not readily erect, and with an ashy grey or whitish tint. In addition, *C. corymbescens* is hardly blackening (melanotic) at base, whereas *C. pseudofissa* is clearly blackening.

The phylogenetic analyses by Stenroos et al. (2002) demonstrated that *C. corymbescens* was not closely related to the species of the *C. furcata* group (*C. farinacea* (Vain.) A. Evans, *C. furcata*, *C. multiformis* G. Merr., and *C. scabriuscula* (Delise) Nyl.); it seemed to be related to *C. petrophila* R.C. Harris and *C. apodocarpa* Robbins instead. The phylogenetic study of *Cladonia* in Thailand by Parnmen et al. (2008) grouped one specimen corresponding to *C. pseudofissa* with *C. rudis* Ahti & Parnmen. Morphologically this species is closely related to the species of *C. furcata* (Ahti et al. 2008). Our present phylogenetic analyses do not allow conclusions on the phylogenetic relationships of *C. corymbescens* and *C. pseudofissa*. Until further *Cladonia* species are included in the phylogenetic analyses, the placement of these species will remain uncertain. The ML and Bayesian analyses suggest that they might be related, but this relationship is not supported, and the branches in both analyses are very long. The MP analysis groups *Cladonia pseudofissa* with *C. ceratophylla* and *C. pityrophylla*, but this relationship is not well supported either. A denser sampling of taxa is needed to clarify the phylogenetic relationships between the species.

As far as is known, the geographic ranges of *C. corymbescens* and *C. pseudofissa* do not overlap; *C. corymbescens* is primarily Australasian to Melanesian and *C. pseudofissa* is Himalayan in distribution. However, the *Cladonia* flora of Indonesia is very poorly known.

Various data support *C. pseudofissa* as a species distinct from *C. corymbescens*. The phylogenetical analyses based on two independent loci separate *Cladonia pseudofissa* and *C. corymbescens* into two well-supported clades, clades that are also supported in the single gene analyses (data not shown). According to the genealogical concordance in phylogenetic species recognition (Taylor et al. 2000), these clades represent different species. In addition, morphological and distributional differences confirm that they are two different taxa.

Acknowledgments

We are grateful for fresh material of *Cladonia* collected by Dr. Maarten Christenhusz (London) in New Caledonia. We are also grateful to Dr. Philippe Clerc (Geneva), who sent our herbarium (H) a large collection of *Cladonia* from the Himalayas. Dr. Jaana Haapala helped us in photographing. Our sincere thanks to Dr. A. R. Burgaz and Dr. M. Piercey-Normore for their valuable comments to improve the manuscript.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.105

Volume 130, pp. 105–130 *January–March 2015*

Morphology and phylogeny of four new *Lactarius* species from Himalayan India

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Abstract —Four new species of *Lactarius* are described from Himalayan India. *Lactarius olivaceoglutinus, L. pyriodorus,* and *L. yumthangensis* belong to *L.* subg. *Piperites* and *L. indochrysorrheus* is closely related to some representatives of *L.* subg. *Russularia.* An ITS based phylogeny confirms the phylogenetic placement of the four new species, although the monophyly of neither *Lactarius* subg. *Piperites* nor *L.* subg. *Russularia* can be confirmed. The ITS data also suggest that the Indian species are closely related to some European and American species.

Key words — ectomycorrhizal fungi, macrofungi, *Russulaceae*, Sikkim

Introduction

After segregation of the well-known ectomycorrhizal milkcaps into *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel (Buyck et al. 2008, 2010), *Lactarius* sensu novo comprises three subgenera (Verbeken & Nuytinck 2013): *L.* subg. *Piperites* (Fr. ex J. Kickx f.) Kauffman, *L.* subg. *Russularia* (Fr. ex Burl.) Kauffman, and *L.* subg. *Plinthogali* (Burl.) Hesler & A.H. Sm. *Lactarius* subg. *Piperites* can be characterized as follows: pileus sticky to slimy/glutinous (more rarely dry and shiny), often hairy and/or zonate or with watery spots; stipe dry or sticky, often scrobiculate (Heilmann-Clausen et al. 1998, Basso 1999). In contrast, representatives of *L.* subg. *Russularia* typically have dry (rarely somewhat sticky) caps and stipes and colours that are predominantly orange or various tinges of brown (Heilmann-Clausen et al. 1998).

Sikkim, a small (0.22% of the land surface) state in India, lies in Eastern Himalaya, which is part of the Himalaya Hotspot, one of the 34 Global Biodiversity Hotspots (www.biodiversityhotspots.org). Within a very small geographical area it is substantially diverse in flora, fauna, and mycobiota, the latter seriously underexplored. During mycological expeditions to different temperate to subalpine areas of the North District of Sikkim in 2009 (KD & AV), 2011 (KD), and 2012 (KD), we collected ectomycorrhizal (ECM) fungi, with *Lactarius* s.l. one of the dominant ECM genera in Dombang, Shingba Rhododendron Sanctuary, and Zema. Dombang is a subalpine coniferous to mixed (coniferous & broadleaf) forest dominated by *Picea spinulosa*, *Abies densa*, *Tsuga dumosa*, and *Larix griffithii*. Shingba Rhododendron Sanctuary contains subalpine coniferous and mixed forests distributed in Yumthang valley and its adjoining areas; apart from several *Rhododendron* species, this protected area is dominated by *Abies densa*, *Picea spinulosa*, *Tsuga dumosa, Larix griffithii*, *Magnolia globosa*, *M. campbellii*, *Acer pectinatum*, and *Betula utilis*. Zema is a subalpine coniferous forest dominated by *Abies densa*.

Although Sikkim harbours a large number of russulaceous taxa, only 32 species (out of ca 210 taxa recorded from India) have been reported (Berkeley 1852; Das 2009; Das et al. 2010, 2013; Das & Verbeken 2011, 2012; Van de Putte et al. 2012). We describe here three new species of *Lactarius* subg. *Piperites* (*L. olivaceoglutinus, L. pyriodorus*, and *L. yumthangensis*) and one new species of *L.* subg. *Russularia* (*L. indochrysorrheus*).

Materials & methods

Morphological study

Macromorphological characters were described from the fresh basidiomata in daylight. Colour codes and terms follow the Colour identification chart of British Fungus Flora (Henderson et al. 1969, here prefixed by "a:") or the Methuen Handbook of Colour (Kornerup & Wanscher 1981, here prefixed by "b:"). Spore print colour codes follow Kränzlin (2005, here prefixed by "c:").

Micromorphological structures such as basidia, hymenial cystidia, pileipellis, stipitipellis, etc. were observed from free-hand sections of dry samples mounted in a mixture of 5% KOH, 30% Glycerol, Phloxine, and Cotton Blue using an Olympus CX41 compound microscope. Spores and spore ornamentation were studied in Melzer's reagent. Drawings were made from SEM images obtained in different magnifications. Spore measurements were calculated based on 20 basidiospores per specimen; dimensions represent minimum–**mean**–maximum length × minimum–**mean**–maximum width, and Q = length/width ratio. Herbarium names are after Holmgren et al. (1990).

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried fruiting bodies according to Nuytinck & Verbeken (2003) with slight modifications (Van de Putte et al. 2010). The internal transcribed

Table 1. Collections of *Lactarius* and allied species used for molecular analyses.

Species	Voucher	Herbarium	Origin	Genbank
Lactarius akahatsu	Verbeken 04-141	GENT	Thailand	KF133269
L. albocarneus	Verbeken 98-071	GENT	France	KJ742389
	Verbeken 98-080	GENT	France	KF241545
L. alboscrobiculatus	Le 175	CMU, SFSU, GENT	Thailand	EF141538
L. aspideus	Walleyn 3815	GENT	Czech Rep.	KJ742390
L. atroviridis	Verbeken 05-306	GENT	USA	KF133270
L. auriolla	Walleyn 1601	GENT	Sweden	KF133257
L. indochrysorrheus	KD 11-002 (Holotypus)	BSHC, GENT	India	KJ742391
L. azonites	Verbeken 00-124	GENT	Belgium	KF241540
L. brunneoviolaceus	Verbeken 04-220	GENT	France	KJ742392
	Verbeken 04-249	GENT	France	KJ742393
	Walleyn 1605	GENT	Sweden	KJ742394
	Eberhardt 24.08. 04-8	UPS	Sweden	KJ742395
L. caespitosus				FJ845421
L. camphoratus	Oberwinkler 46773	TUB	Germany	AY606945
L. chichuensis	Wang 1236	HKAS	China	KF475766
L. chrysorrheus	Nuytinck 01-089	GENT	Belgium	KJ742396
	D'Hooge 08-020	GENT	France	KJ742397
	Eberhardt 04.10. 02-8	UPS	Italy	KF133261
L. aff. *chrysorrheus*	Verbeken 04-212	GENT	USA	KJ742398
	Verbeken 05-359	GENT	USA	KJ742399
L. citriolens	Eberhardt 20.09.04-3	UPS	Sweden	DQ422003
L. controversus	Verbeken 00-117	GENT	Italy	KF241544
L. crassiusculus	Le 369	CMU, SFSU	Thailand	EF560684
L. cyathuliformis	Eberhardt 04.09.04-3	UPS	Sweden	KF133266
L. flexuosus	Walleyn 2136	GENT	Sweden	KJ742400
	Walleyn 3178	GENT	Czech Rep.	KJ742401
	Eberhardt 06.09.02-1	UPS	Sweden	DQ421992
L. formosus	Le 382 (Holotypus)	CMU, SFSU, GENT	Thailand	EF141549
L. fuliginosus	Basso 97-24	priv. herb.	Sweden	JQ446111
L. helvus	Eberhardt 08.09. 04-1	UPS	Sweden	KF133263
L. lilacinus	Walleyn 3774	GENT	Belgium	KF133275
L. luridus	Taylor 2003066	UPS	Sweden	KJ742402
	Eberhardt 17.09.04-3	UPS	Sweden	KJ742403
	Eberhardt 10.10. 04-4	UPS	Sweden	KJ742404
	Berteloot 11-011	GENT	Belgium	KF241547
	Berteloot 11-012	GENT	Belgium	KJ742405
	Taylor 2004254	UPS	Sweden	KJ742406
	Walleyn 1455	GENT	Belgium	KJ742407
L. montoyae	KD 1065 (Holotypus)	BSD	India	EF560673
L. necator	Verbeken 04-231	GENT	France	KF133276
L. olivaceoglutinus	KD 11-103 (Holotypus)	BSHC, GENT	India	KJ742408
L. pallescens				DQ974747
L. peckii	Nuytinck 04-020	GENT	USA	KF133277
L. pseudouvidus	Eberhardt 24.08. 04-14	UPS	Sweden	KJ742409
	Eberhardt 24.08. 04-13	UPS	Sweden	KJ742410

L. pubescens	Eberhardt 15.09.02-2	UPS	Sweden	DQ421996
L. pyriodorus	KD 11-027 (Holotypus)	BSHC, GENT	India	KJ742411
L. quieticolor	Eberhardt 10.09.04-1	UPS	Sweden	DQ422002
L. quietus	Eberhardt 16.09.04-6	UPS	Sweden	KF133264
L. rufus	Nuytinck 02-008	GENT	Norway	KF241543
L. sphagneti	Walter 083	TUB	Germany	KJ742412
L. subdulcis	Vervisch 06-024	GENT	Belgium	KF133279
L. subplinthogalus	Verbeken 04-219	GENT	USA	KF241539
L. subsericatus	Eberhardt 11.10.04-8	UPS	Sweden	DQ422011
L. thyinos	Voitk 23-08-04	priv. herb.	Canada	KF133271
L. torminosus	Walleyn 3183	GENT	Czech Rep.	KF133281
L. trivialis	Van de Putte 10-011	GENT	Russia	KJ742413
	Walleyn 3179	GENT	Czech Rep.	KJ742414
	Eberhardt27.08. 02-17a	UPS	Sweden	DQ421991
L. uvidus	Van de Putte 10-027	GENT	Russia	KF241546
	Nuytinck 01-033	GENT	Finland	KJ742415
	Walleyn 1237	GENT	France	KJ742388
	Walleyn 2119	GENT	Sweden	KJ742416
	Eberhardt 28.08. 02-24	UPS	Sweden	KJ742417
L. vietus	Eberhardt 11.10.04-1	UPS	Sweden	KF133267
L. vinaceorufescens	Nuytinck 07-018	GENT	Canada	KF241542
L. yumthangensis	KD 11-147 (Holotypus)	BSHC, GENT	India	KJ742418
Lactifluus volemus	Eberhardt 09.08. 04-5	UPS	Sweden	DQ422008
Lactifluus vellereus	Eberhardt 20.09.04-22	UPS	Sweden	DQ422034
Multifurca ochricompacta	Buyck 02-107	PC	USA	DQ421984
M. zonaria	Desjardin 7442	SFSU, PC, BBH	Thailand	DQ421990
Russula cyanoxantha	Eberhardt 29.09. 02-2	UPS	France	DQ422033
R. nigricans	Eberhardt 20.09.04-7	UPS	Sweden	DQ422010

spacer region of the nuclear ribosomal DNA (ITS) was amplified and sequenced using primers ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993). PCR amplification protocols follow Le et al. (2007); sequencing was conducted with an ABI 3730XL or ABI 3700 by Macrogen (Amsterdam, The Netherlands). Sequences were assembled and edited with Sequencher™ v4.9 (GeneCodes Corporation, Ann Arbor, Michigan, U.S.A.). Specimens and sequences used in the phylogenetic analysis are shown in Table 1.

Alignment and phylogenetic analyses

Alignment was conducted with the on-line version of MAFFTv7 (Katoh & Standley 2013), applying the E-INS-I strategy, a very slow method recommended for fewer than 200 sequences with multiple conserved domains and long gaps. The alignment was manually refined in BioEdit v7.0.9.0 (Hall 1999). Ambiguously aligned positions were detected using Gblocks v0.91b (Castresana 2000), specifying less stringent conditions than default. The minimum number of sequences for a conserved position and for a flank position was set to half the number of sequences, the number of contiguous non-conserved positions was set to 10, and the minimum length of a block after gap cleaning to 5 and positions with gaps were not treated differently from other positions. The ITS

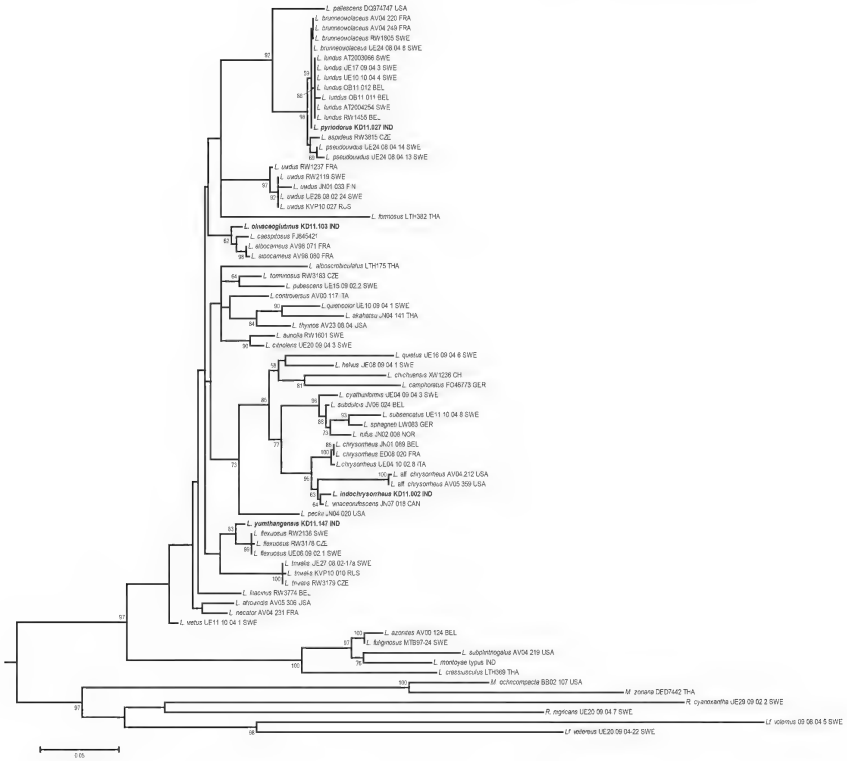
L. pallescens DQ374747 USA
L. brunneoviolaceus AV04 220 FRA
L. brunneoviolaceus AV04 249 FRA
L. brunneoviolaceus RW1905 SWE
L. brunneoviolaceus UE24 08 04 5 SWE
L. lundus AT2003966 SWE
L. lundus UE17 09 04 3 SWE
L. lundus UE10 10 04 4 SWE
L. lundus OB11 012 BEL
L. lundus OB11 011 BEL
L. lundus AT2004254 SWE
L. lundus RW1409 BEL
L. pyriodorus KD11.927 IND
L. asodes RW3815 CZE
L. pseudouvidus UE24 08 04 14 SWE
L. pseudouvidus UE24 08 04 13 SWE
L. uvidus RW1237 FRA
L. uvidus RW2119 SWE
L. uvidus JN01 033 F-N
L. uvidus UE26 08 02 24 SWE
L. uvidus KVP10 027 RUS
L. flavidus LTH382 THA
L. olivaceoglutinus KD11.103 IND
L. caespitosus FJ845421
L. allocarneus AV98 071 FRA
L. azonicarneus AV98 093 FRA
L. atlassambuloides LTH175 THA
L. tominosus RW3183 CZE
L. pubescens UE16 09 02 2 SWE
L. controversus AV00 117 TA
L. quercicolor UE10 09 04 1 SWE
L. sharattsu JN84 141 THA
L. thyrsos AV23 08 04 USA
L. aurantia RW1601 SWE
L. citriolens UE20 09 04 3 SWE
L. quietus UE16 09 04 6 SWE
L. helvus JE08 09 04 1 SWE
L. chichuensis XW1236 CH
L. camphoratus FO46773 GER
L. cyathuliformis UE04 09 04 3 SWE
L. subdulcis JV06 024 BEL
L. subcircatus UE11 10 04 8 SWE
L. tomentosus LW085 GER
L. rufus JN02 008 NOR
L. chrysorrheus JN01 069 BEL
L. chrysorrheus EO08 020 FRA
L. chrysorrheus LE04 10 02 8 ITA
L. aff. chrysorrheus AV04 212 USA
L. aff. chrysorrheus AV05 359 USA
L. indochrysorrheus KD11.902 IND
L. vinaceorufescens JN07 018 CAN
L. peckii JN04 020 USA
L. yumthangsnea KD11.147 IND
L. flexuosus RW2136 SWE
L. flexuosus RW3178 CZE
L. flexuosus UE06 09 02 1 SWE
L. tiwelis JE27 08 02-17a SWE
L. tiwelis KVP10 010 RUS
L. tiwelis RW3179 CZE
L. flavidus RW3774 BEL
L. affluoridis AV05 306 USA
L. necator AV04 231 FRA
L. vetus UE11 10 04 1 SWE
L. azonites AV00 124 BEL
L. fulginosus MTB97-24 SWE
L. subplinthogalus AV04 219 USA
L. montoyae typus IND
L. crassisuccus LTH369 THA
M. ochrocompacta BB92 107 USA
M. zonaria DED7442 THA
R. cyanoxantha UE29 09 02 2 SWE
R. nigricans UE20 09 04 7 SWE
Lr. volemus 09 08 04 5 SWE
Lr. velereus UE20 09 04-22 SWE
0.05

Plate 1. The obtained ML topology based on ITS sequences of *Lactarius, Multifurca, Russula,* and *Lactifluus* species. Bootstrap values >50% are indicated. Names in bold are the new Indian species described in this paper. The scale bar represents the number of nucleotide changes per site.

sequences were partitioned into 5 partitions: the ribosomal genes 18S, 5.8S, and LSU and the spacer regions ITS1 and ITS2.

Maximum Likelihood (ML) analysis was performed in RAxML v7.0.3 (Stamatakis 2006), combining a ML search with the Rapid Bootstrapping algorithm for 1000 replicates. The model GTRGAMMA was estimated for each partition separately.

Results

Phylogeny

Plate 1 shows the obtained ML topology with bootstrap support (BS) values >50% displayed. The tree shows a well-supported genus *Lactarius* (97% BS). Within *Lactarius, L.* subg. *Plinthogali* receives a high support value (100% BS), but monophyly of neither *Lactarius* subg. *Piperites* nor *L.* subg. *Russularia* (sensu Heilmann-Clausen et al. 1998) is supported. *Lactarius pyriodorus, L. olivaceoglutinus,* and *L. yumthangensis* are closely related to species

traditionally placed in *L.* subg. *Piperites*. *Lactarius indochrysorrheus* is closely related to the European *L. chrysorrheus* Fr. and the North American *L. vinaceo-rufescens* A.H. Sm. and *L.* aff. *chrysorrheus*, which are assigned to *L.* subg. *Russularia* in morphology-based taxonomy.

Species delimitation for this paper is mainly based on morphological features. We were not able to include enough specimens/sequences from the newly proposed species to test species delimitation in the phylogenetic tree.

Taxonomy

Lactarius indochrysorrheus K. Das & Verbeken, **sp. nov.** PLATES 2, 3, 6A,B,E

MYCOBANK MB804887

Differs from *Lactarius chrysorrheus* by its more viscid pileus with its pileipellis an ixocutis covered by a thick gelatinous layer.

TYPE: India. Sikkim: North District, Dombang, 27°43′35.2″N 88°45′15.2″E, alt. 2920 m, 18.VIII.2011, K. Das, KD 11-002 (**Holotype**, BSHC; **isotype**, GENT).

ETYMOLOGY: an Indian look-alike of the European *Lactarius chrysorrheus*.

PILEUS 25–70 mm diam., convex with slightly pubescent and inrolled margin when young, gradually planoconvex to applanate with slightly depressed centre, rarely with a central papilla, sometimes becoming widely infundibuliform; margin decurved with maturity, becoming very irregularly wavy; surface smooth to greasy, viscid (sticky), shiny, brownish orange, pinkish buff to salmon (a: 45) or orange (b: 6A6) (cinnamon (a: 10) to somewhat rusty after maturity), gradually apricot cream to paler up to yellow (a: 5E), towards margin distinctly zonate with several zones over the whole diameter but most dense in the center; zones consisting of darker and watery spots; margin very faintly and shortly striate. LAMELLAE subdecurrent, crowded (18–22/cm at pileus margin), pale yellow (a: 4D) when young, gradually pink-spotted, finally becoming brown to reddish brown, with lamellulae in 9 series; edge entire, concolorous. STIPE 45–65 × 6–8.5 mm, slender, subcylindrical to cylindrical or slightly widened towards base; surface smooth, slightly greasy, strigose (hairy) at base, much paler than the pileus, especially in the upper part (very pale pinkish), very pale salmon to vinaceous, gradually darker up to rust to rusty tawny or pale brick red. CONTEXT hollow in stipe, pinkish yellow (a: 4D) to pale salmon, turning lemon yellow (a: 54) to greenish yellow (a: 57) after cut, changing to salmon (a: 45) with $FeSO_4$, and greenish with Guaiac. LATEX abundant, white, turning quickly to greenish yellow. TASTE first mild, then becoming bitter and acrid. ODOUR not distinctive. SPORE PRINT pale cream (c: 10 Y).

BASIDIOSPORES 6.4–**7.4**–8.5 × 5.6–**6.2**–6.9 µm, (Q = 1.08–**1.18**–1.33), subglobose to ellipsoid; ornamentation amyloid, ≤0.9 µm high, composed of conical to spine-shaped warts, with short or medium, irregular to regular

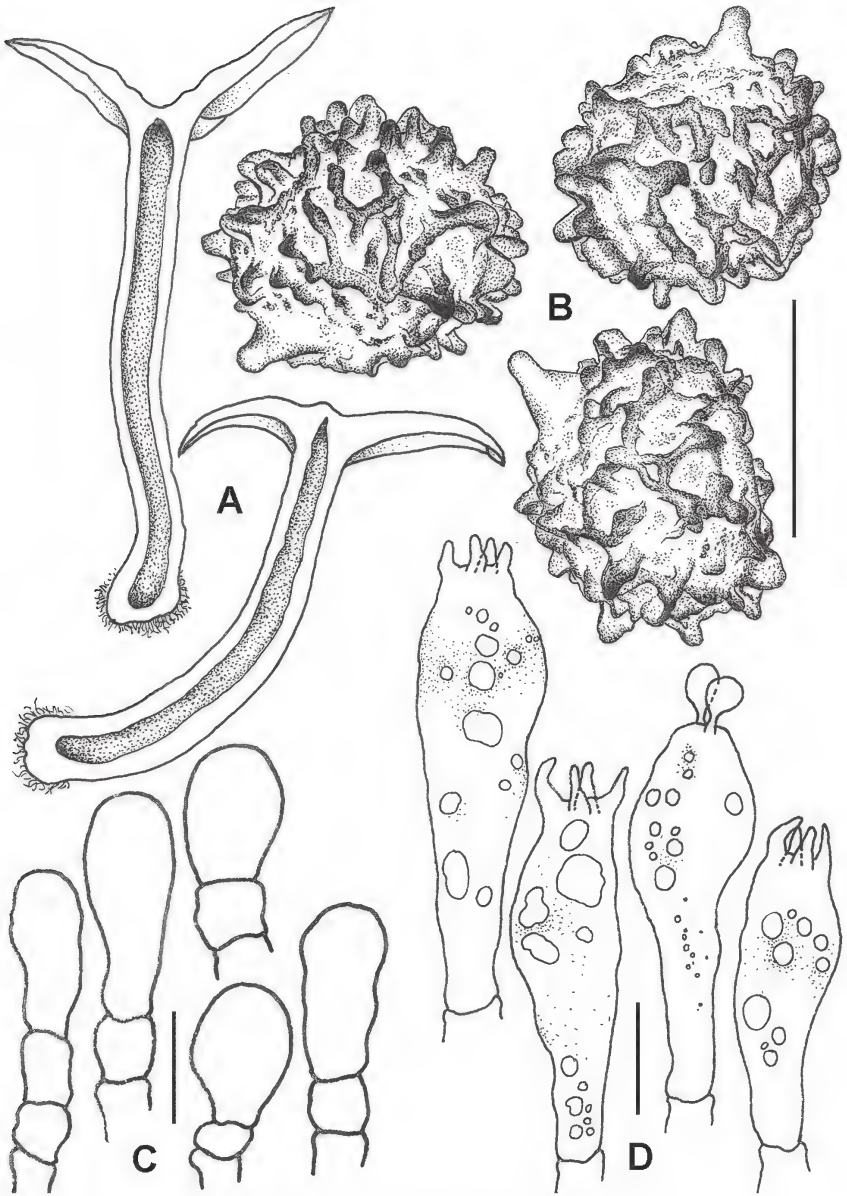

Plate 2: *Lactarius indochrysorrheus* (Holotype KD 11-002).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Marginal cells. D. Basidia. Scale bars: B = 5 µm; C, D = 10 µm.

ridges which are aligned or connected and forming a partial to incomplete reticulum; some isolated small warts present; plage sometimes distinct and amyloid. Basidia 30–44 × 9–13 µm, 4-spored, subclavate to ventricose; sterigmata 2.5–4 × 1.5–2 µm. Pleuromacrocystidia 32–70 × 7.5–11 µm, fairly abundant, emergent ≤10–30 µm, narrowly clavate, cylindric with tapered apex or fusoid, often with mucronate to subcapitate apex, slightly thick-walled (wall ≤0.5 µm); content refractive. Pleuropseudocystidia filamentous, 3–5 µm wide. Lamellar edge fertile with basidia, cystidia and marginal cells. Cheilomacrocystidia 35–55 × 8–9 µm, moderately abundant, subclavate to narrowly clavate or ventricose to fusoid sometimes with mucronate apex; content refractive. Cheilopseudocystidia filamentous. Marginal cells 11–20 × 7–11 µm, mostly clavate to subclavate, often multiseptate. Hymenophoral trama with lactifers. Pileipellis an ixocutis, ≤170 µm thick, with gelatinous layer mostly extended 10–15 µm beyond the hyphal layer; hyphae repent to suberect, ≤3.5 µm wide, branched, septate. Stipitipellis an ixocutis, composed of repent hyphae mostly in parallel pattern; hyphae ≤3.5 µm wide, branched, septate. Stipe trama mostly with numerous nested sphaerocytes. Clamp connections absent.

Ecology & Distribution — Gregarious under *Abies densa* and *Picea spinulosa* in subalpine coniferous or mixed (coniferous and broadleaf) forest. August. Fairly common.

Additional specimens examined: **INDIA**. **Sikkim**: North District, Dombang, 27°43′35.2″N 88°45′15.2″E, alt. 2920 m, 14.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 032 (BSHC, GENT); North District, Shingba Rhododendron sanctuary, 27°43′42.1″N 88°44′58.8″E, alt. 2889 m, 24.VIII.2011, K. Das, KD 11-082 (BSHC); 27°44′03.5″N 88°44′23.3″E, alt. 3208 m, 26.VIII.2012, K. Das, KD 11-107 (BSHC).

Notes — Zonate species with similar cap colours and milk that stains quickly sulphur to greenish yellow are European *L. chrysorrheus*, North American *L. vinaceorufescens,* and an undescribed American species [labeled *L.* aff. *chrysorrheus* in the phylogenetic tree and Table 1]. ITS sequence comparison suggests the new species described here is close to the three species but not conspecific.

Lactarius chrysorrheus is less sticky, which is reflected in the pileipellis structure, a loosely interwoven cutis without distinct slime layer (Heilmann-Clausen et al. 1998). *Lactarius vinaceorufescens* differs by a pileipellis with a relatively thin ixocutis and the lack of ixocutis in the stipitipellis (Hesler & Smith 1979). *Lactarius xanthogalus* Verbeken & E. Horak, described from Papua New Guinea (Verbeken & Horak 2000), has a zonate orange to paler cap and latex that becomes immediately sulphur yellow but clearly differs by the zebroid ornamented spores.

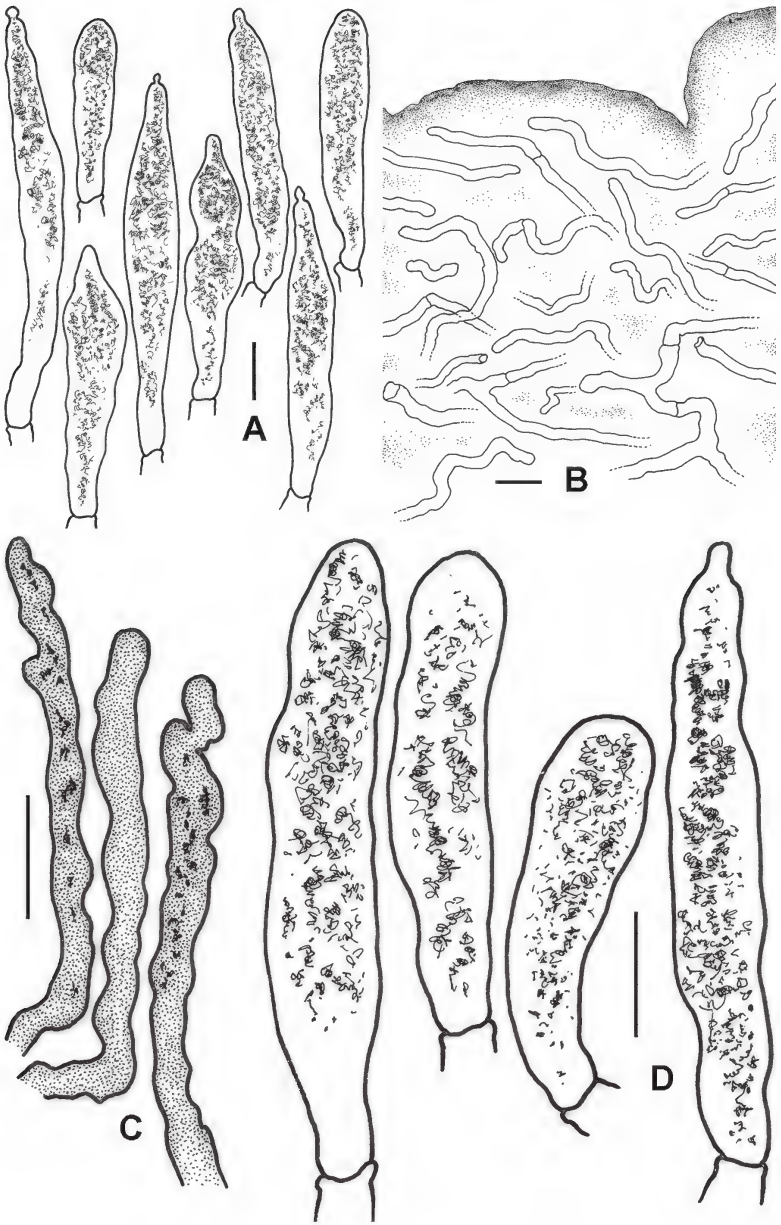

Plate 3. *Lactarius indochrysorrheus* (Holotype KD 11-002).
A. Pleuromacrocystidia. B. Radial section through pileipellis.
C. Pleuropseudocystidia. D. Cheilomacrocystidia. Scale bars = 10 µm.

Lactarius olivaceoglutinus K. Das & Verbeken, **sp. nov.** PLATES 4, 5, 6C,D,F

MYCOBANK MB 804888

Differs from *Lactarius albocarneus* by its olivaceous pileus and its watery white latex that turns faintly pinkish on bruised or cut gill tissue.

TYPE: India. Sikkim: North District, Shingba Rhododendron sanctuary, 27°44′19.5″N 88°44′25.9″E, alt. 3252 m, 26.VIII.2011, K. Das, KD 11-103 (**Holotype**, BSHC; **isotype**, GENT).

ETYMOLOGY: named after the olive and extremely glutinous pileus of the basidiocarps.

PILEUS 40–68(–120) mm diam., at first convex, gradually becoming planoconvex with slightly depressed centre; surface smooth, viscid to highly glutinous, olive yellow (b: 3D8) to olive brown (b: 4E8, 4F6) or golden brown (b: 5D7) to greenish grey, grey-olive or darker towards centre and paler margin, azonate; margin non-striate, incurved when young, decurved with maturity. LAMELLAE adnexed to broadly adnate, crowded (17–18/cm at margin), with lamellulae in 5–6 series, pale cream to cream; edge entire, concolorous. STIPE 40–75 × 12–17 mm, clavate to subclavate (broader towards base) or ventricose (narrower towards apex and base); surface smooth or sometimes with shallow depressions (but without scrobicules), sticky, pale salmon to pale yellow (b: 3A3 to 4A3), gradually whitish towards base; base strigose, pale cream to white, sometimes with ochraceous spots. CONTEXT firm, stuffed to hollow in stipe, cream (a: 4D) to pale cream, unchanging when exposed or with KOH, changing to olivaceous (a: 62) to leaf green (a: 59) with Guaiac and pale green with $FeSO_4$. LATEX whitish, very watery, turning faintly pinkish on bruised or cut lamellae. TASTE mild and agreeable or slightly acrid. ODOUR indistinct. SPORE PRINT pale yellow (c: 30Y, 2M).

BASIDIOSPORES 8.0–**9.3**–10.1(–11) × 6.7–**7.3**–7.8(–8.4) µm, [Q = 1.16–**1.28**–1.33(–1.44)], broadly ellipsoid to ellipsoid; ornamentation amyloid, ≤1.2 µm high, composed of narrow, low and high ridges aligned and parallel, forming zebroid pattern, some connected with adjacent ones (but never forming a true reticulum), with each high ridge bearing elongated to conical (with rounded apex) warts; plage indistinct. BASIDIA 40–60 × 11.5–13 µm, 2–4-spored, ventricose; sterigmata 6–9 × 2–2.5 µm. PLEUROMACROCYSTIDIA 57–88 × 8–12 µm, abundant, subcylindric or subclavate to fusiform with rounded, mucronate to moniliform (rarely) apex, emergent ≤41 µm; content dense. PLEUROPSEUDOCYSTIDIA cylindrical to tortuous, rare, never emergent, 4–5 µm wide; content refringent. LAMELLAR EDGE sterile with marginal cells and some cystidia. CHEILOMACROCYSTIDIA 32–57 × 7–9 µm, subcylindrical to subfusiform, mostly with rounded apex. MARGINAL CELLS 13–23 × 6.5–9 µm, narrowly clavate to clavate or subcapitate, slightly thick-walled, hyaline. HYMENOPHORAL TRAMA with lactifers. PILEIPELLIS an ixotrichoderm, very thick (often variable in thickness), 220–700 µm thick, composed of erect,

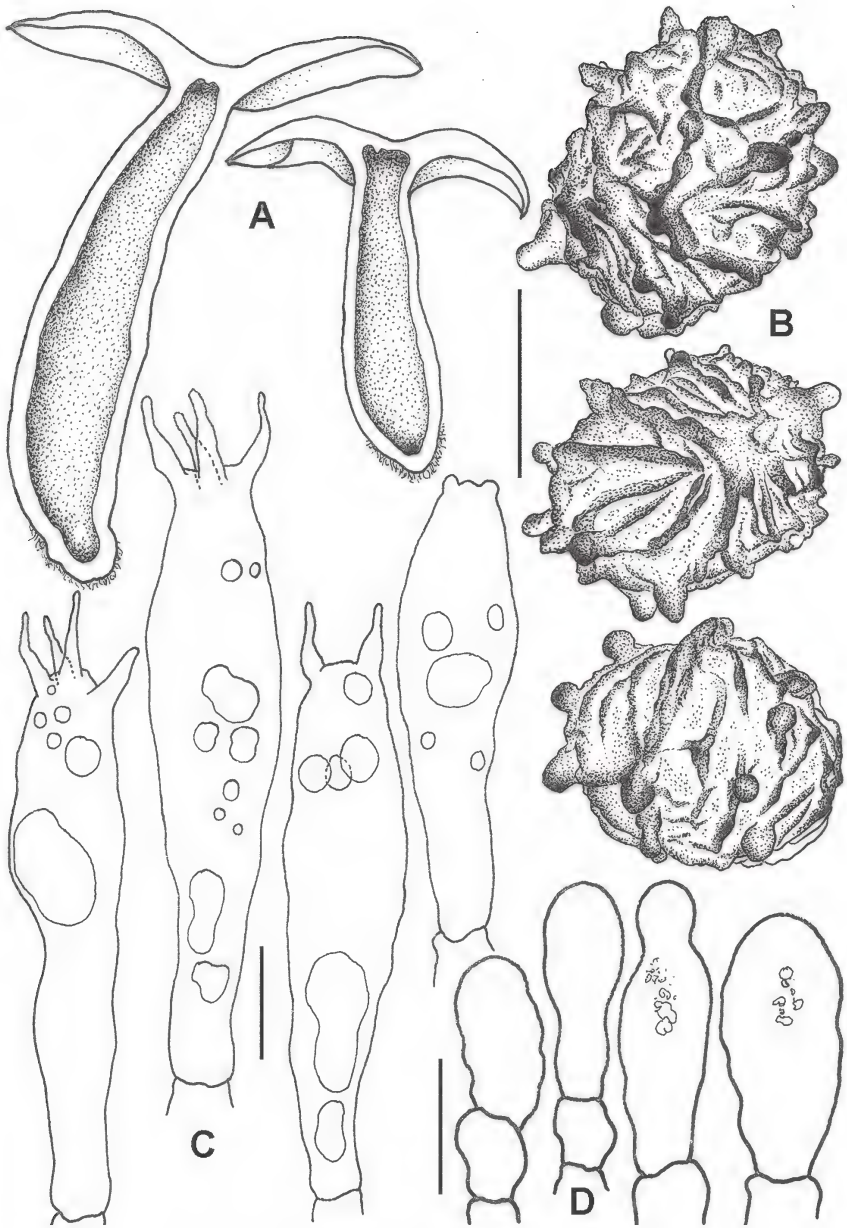

Plate 4. *Lactarius olivaceoglutinus* (Holotype KD 11-103).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Basidia. D. Marginal cells. Scale bars: B = 5 µm; C, D = 10 µm.

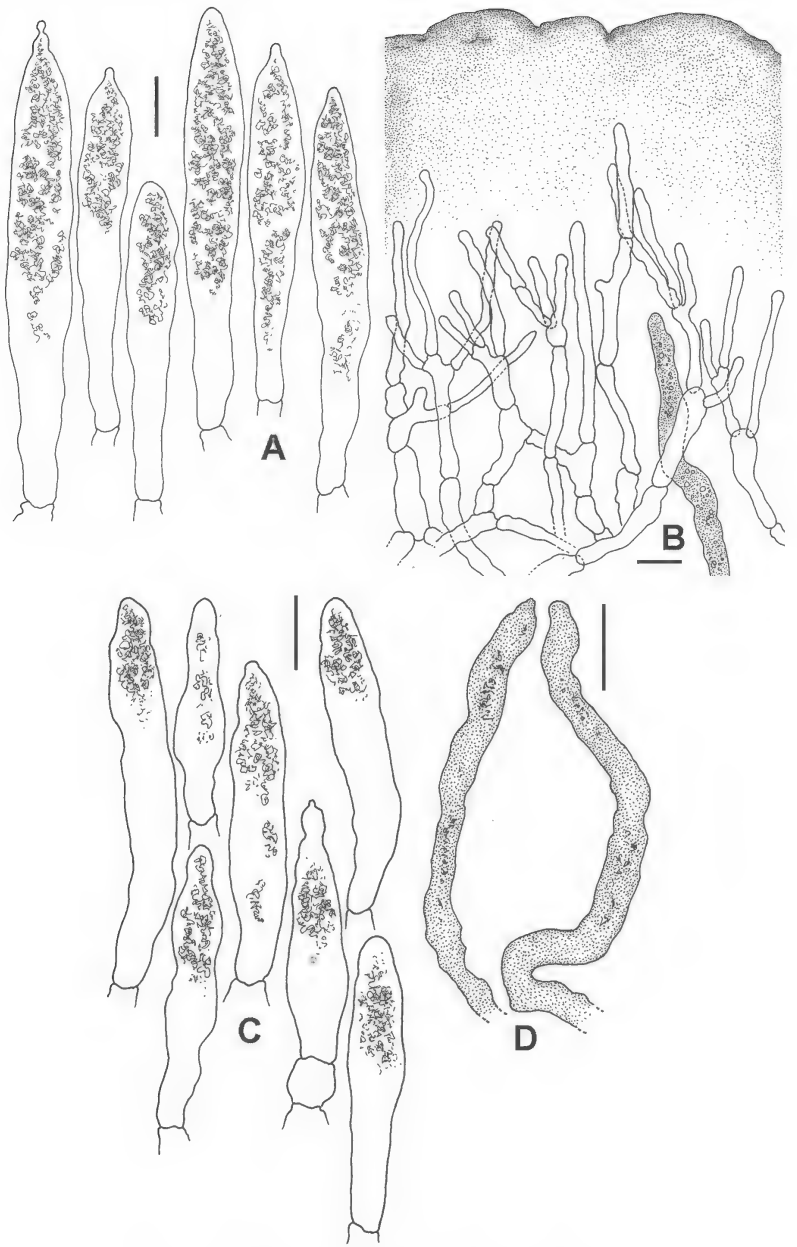

Plate 5. *Lactarius olivaceoglutinus* (Holotype KD 11-103).
A. Pleuromacrocystidia. B. Radial section through pileipellis.
C. Cheilomacrocystidia. D. Pleuropseudocystidia. Scale bars = 10 µm.

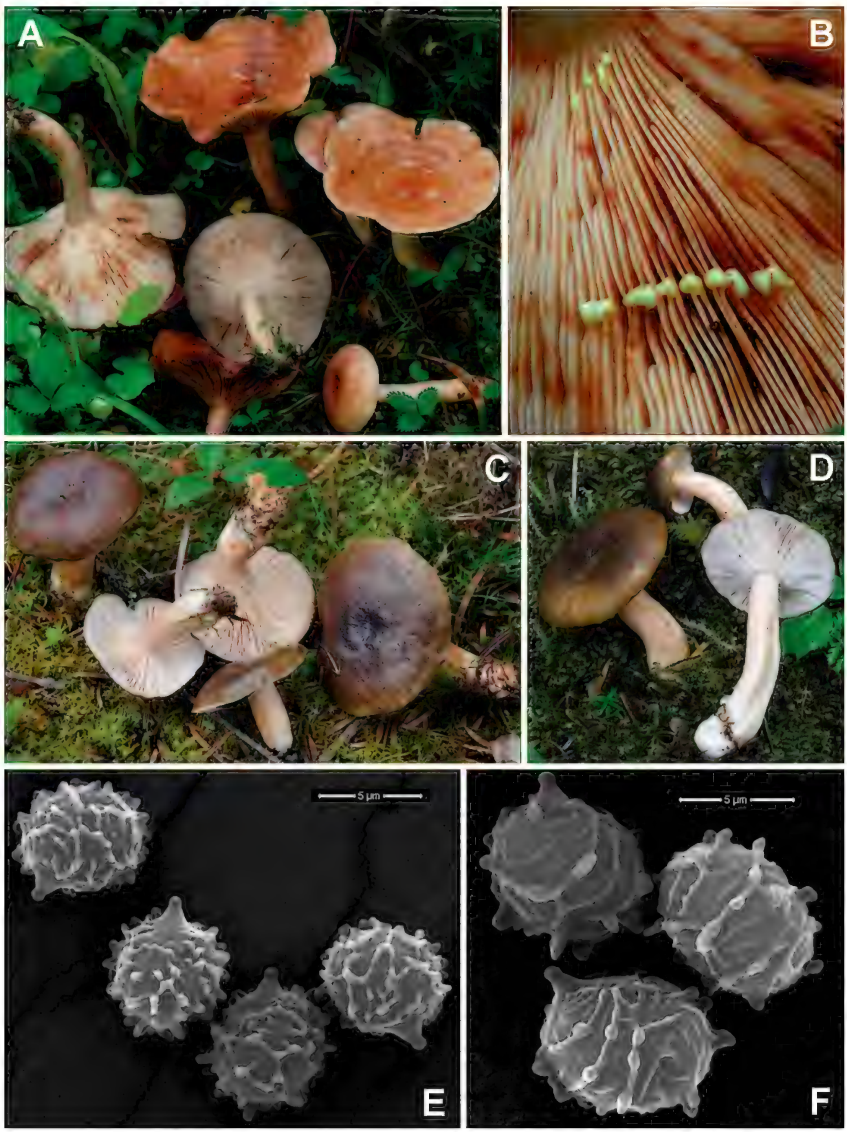

Plate 6. ***Lactarius indochrysorrheus***. A. Fresh basidiomata. B. Latex oozing from cut lamellae. E. Basidiospores (SEM). ***Lactarius olivaceoglutinus***. C, D. Fresh basidiomata. F. Basidiospores (SEM). Scale bars: E, F = 5 µm.

branched multi-septate hyphae (≤3.5 µm wide) and few lactifers embedded in a slime layer of variable thickness underlying somewhat repent hyphae. Stipitipellis an ixotrichoderm, ≤120 µm thick, composed of erect branched

septate hyphae (≤3 µm wide) embedded in a slime layer. Stipe trama with numerous nested sphaerocytes. Clamp connections absent.

Ecology & Distribution — Gregarious under *Abies densa* in subalpine coniferous or mixed (coniferous and broadleaf) forest. August–September. Fairly common.

Additional specimens examined: **India. Sikkim**: North District, Shingba Rhododendron sanctuary, 27°45′11.1″N 88°43′47.5″E, alt. 3580 m, 29.VIII.2011, K. Das, KD 11-144 (BSHC); North District, Dombang, 27°44′53.1″N 88°44′58.8″E, alt. 3058 m, 03.IX.2011, K. Das, KD 11-191 (BSHC); 27°44′51″N 88°46′32″E, alt. 2940 m, 15.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 09-049 (BSHC, GENT).

Notes — The combination of the viscid to glutinous pileus and stipe and the ixotrichoderm pileipellis clearly places the present species in *L.* sect. *Pallidini*. *Lactarius olivaceoglutinus* can be recognized by its distinctly glutinous grey-olive to greenish grey pileus, pale salmon to pale yellow sticky stipe, whitish watery latex that turns the bruised lamellae faintly pinkish, large spores with mostly zebroid patterns of ridges (that carry warts), and its occurrence under *Abies* sp. ITS sequences and morphology (mainly the thick pileipellis ixotrichoderm) suggest a close relationship with the European *L. albocarneus* Britzelm. [= *L. glutinopallens* F.H. Møller & J.E. Lange] and North American *L. caespitosus* Hesler & A.H. Sm. The pileus of *L. albocarneus* however, is dominantly cream to at most olivaceous buff and its latex slowly turns to sulfur-yellow (Heilmann-Clausen et al. 1998, Kränzlin 2005) and its spore ridges never bear elongated to conical warts. *Lactarius caespitosus* also has a viscid cap with similar grey to olive brown colours, but its spores are slightly larger with a distinctly low and non-zebroid ornamentation (Hesler & Smith 1979).

In Sikkim *L. elaioviscidus* K. Das & Verbeken is another species with a glutinous olivaceous pileus and watery white latex (Das & Verbeken 2011); however, it is distinguished from *L. olivaceoglutinus* by the presence of a papilla, its greyish yellow to yellowish white stipe without pinkish tinges, latex changing to yellow-yellowish green after long exposure, and smaller basidiospores without zebroid ornamentation (7.0–**8.1**–8.7 × 6.0–**6.7**–7.1 µm).

Lactarius pyriodorus K. Das & Verbeken, **sp. nov.** Plate 7–9, 13a,b,f

MycoBank MB 804889

Differs from *Lactarius formosus* by lacking bundles of glutinous hairs on the pileus.

Type: India. Sikkim: North District, Dombang, 27°44′08.7″N, 88°45′58.3″, alt. 2975 m, 19.VIII.2011, K. Das, KD 11-027 (**Holotype**, BSHC; **isotype**, GENT).

Etymology: in reference to the distinct pear-like odor of the fresh basidiocarps.

Pileus 35–77 mm diam., at first convex with slightly depressed centre, gradually becoming planoconvex with depressed centre and finally broadly to widely infundibuliform, rarely with a small, sometimes blunt central papilla; surface

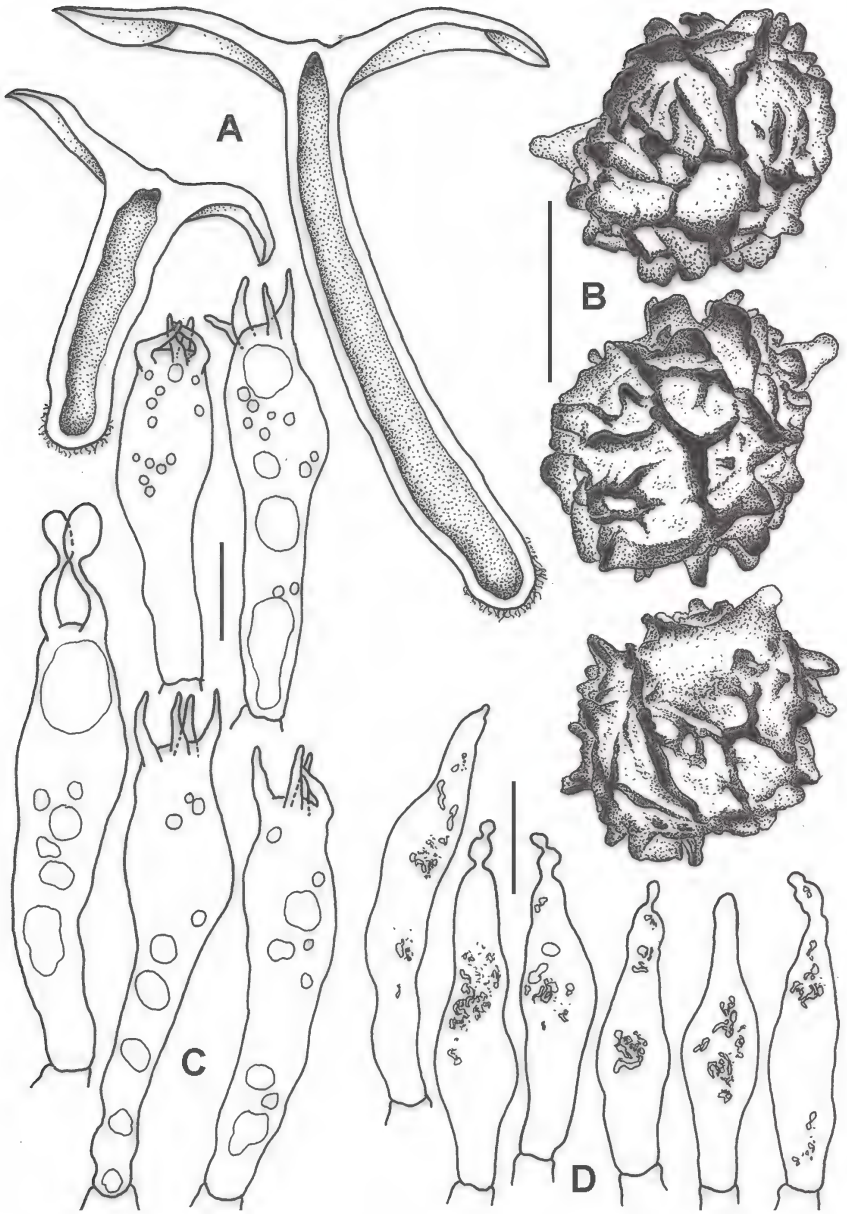

Plate 7. *Lactarius pyriodorus* (Holotype KD 11-027).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Basidia. D. Cheilomacrocystidia. Scale bars: B = 5 µm; C, D = 10 µm.

smooth to finely rugulose under the slimy surface, viscid to highly glutinous and slimy, mostly uniformly colored but sometimes with some indistinct fading zones, bay (a: 19) to umber (a: 18), often sienna (a: 11) towards margin with distinct rust (a: 13) to dark brick (a: 20) or brown vinaceous (a: 25), with broader zonations towards centre and narrower zones towards margin; margin non-striate to very finely and shortly striate, incurved when young, decurved with maturity, becoming very irregularly wavy to undulate. Lamellae broadly adnate to decurrent, crowded (15–16/cm at margin), sometimes forked near the stipe, with lamellulae in 5 series, whitish cream to pale cream, turning livid vinaceous (a: 77) to lilac (a: 79) or darker violet (b: 15D4–5, 15E5) after bruising; edge entire, concolorous. Stipe 35–72 × 10–18 mm, cylindrical or broadest in the middle, narrower towards base, often curved and locally swollen; surface smooth, slightly viscid or sticky, not as viscid as pileus, dingy white to pale cream, buff (a: 52) to vinaceous buff with maturity; base usually paler, strigose. Context firm, rather thick in pileus center and thin in outer half, very soon hollow in stipe, white to pale cream, changing lilac (a: 77; b: 13C3–4) after exposure, leaf green with Guaiac, unchanging with $FeSO_4$ and KOH. Latex white to pale cream, watery, slowly staining the lamellae or context livid vinaceous (a: 77) to lilac (a: 79), unchanging without the contact of lamellae or context. Taste mild at first, becoming slightly bitter and astringent. Odour fruity, very sweet, like pears (as in *Inocybe corydalina* Quél.). Spore print pale cream (c: 10Y).

Basidiospores 8.0–**8.9**–10.5 × 6.9–**7.5**–8.8 µm, (Q = 1.11–**1.18**–1.32), subglobose to broadly ellipsoid, rarely ellipsoid; ornamentation amyloid, ≤1.1 µm high, composed of broad ridges and isolated warts forming a partial to an incomplete reticulum; plage inamyloid or distally amyloid. Basidia 45–52 × 10.5–12 µm, 4-spored, rarely 2-spored, clavate to ventricose; sterigmata 6–7 × 2–2.5 µm. Pleuromacrocystidia 55–80 × 7.5–11 µm, abundant, fusiform with mucronate to moniliform apex, emergent ≤33 µm; content slightly dense. Pleuropseudocystidia cylindric to slightly tortuous, never emergent, 3.5–4 µm wide; content refringent. Lamellar edge sterile. Cheilomacrocystidia 28–43 × 6.5–9 µm, fusiform to lageniform, mostly with moniliform apex. Marginal cells 15–35 × 7–10 µm, cylindric to clavate, slightly thick-walled, hyaline. Subhymenium ≤20 µm thick. Hymenophoral trama with abundant lactifers. Pileipellis an ixocutis to ixotrichoderm, ≤320 µm thick, composed of few repent hyaline hyphae and abundant erect hyphae (2–4 µm broad). Clamp connections absent.

Ecology & Distribution — Gregarious to caespitose under *Abies densa* and *Tsuga dumosa* in subalpine mixed (coniferous and broadleaf) forest. August. Fairly common.

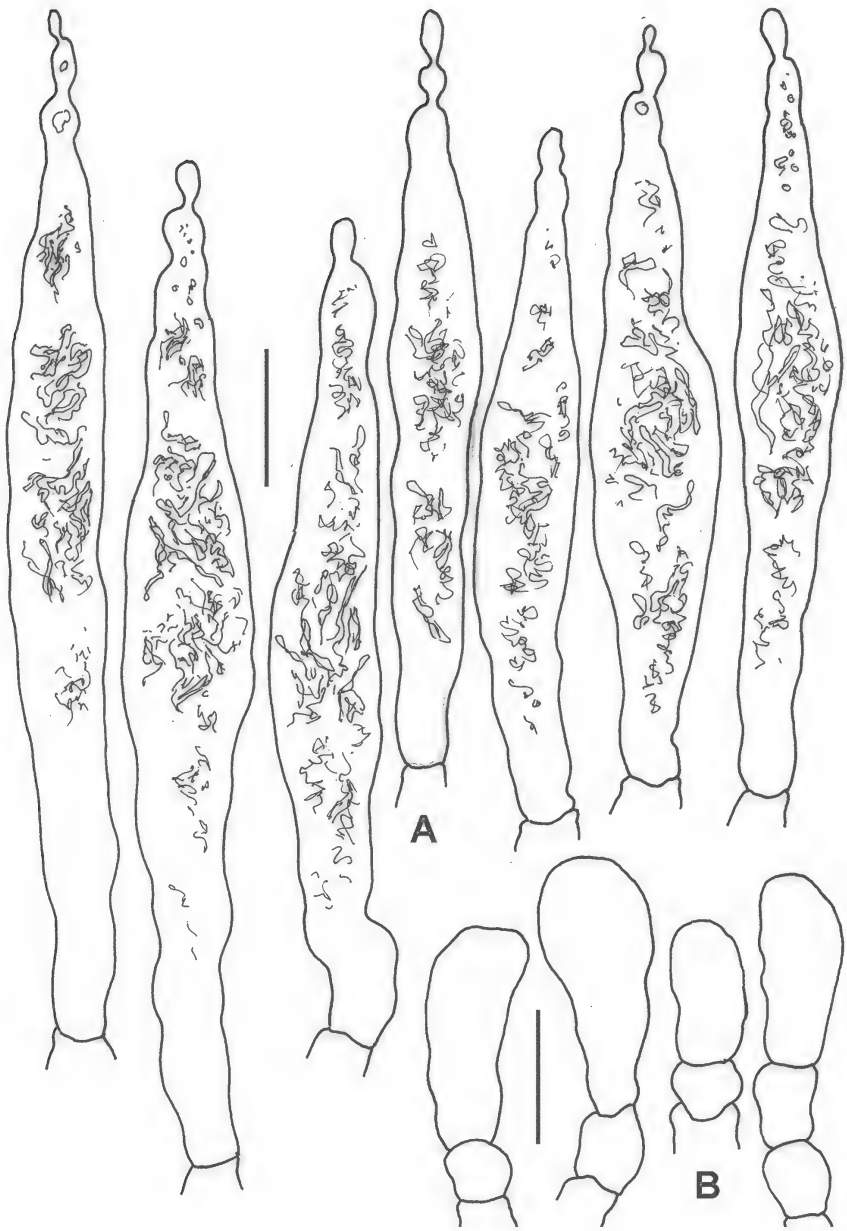

Plate 8. *Lactarius pyriodorus* (Holotype KD 11-027).
A. Pleuromacrocystidia. B. Marginal cells. Scale bars = 10 µm.

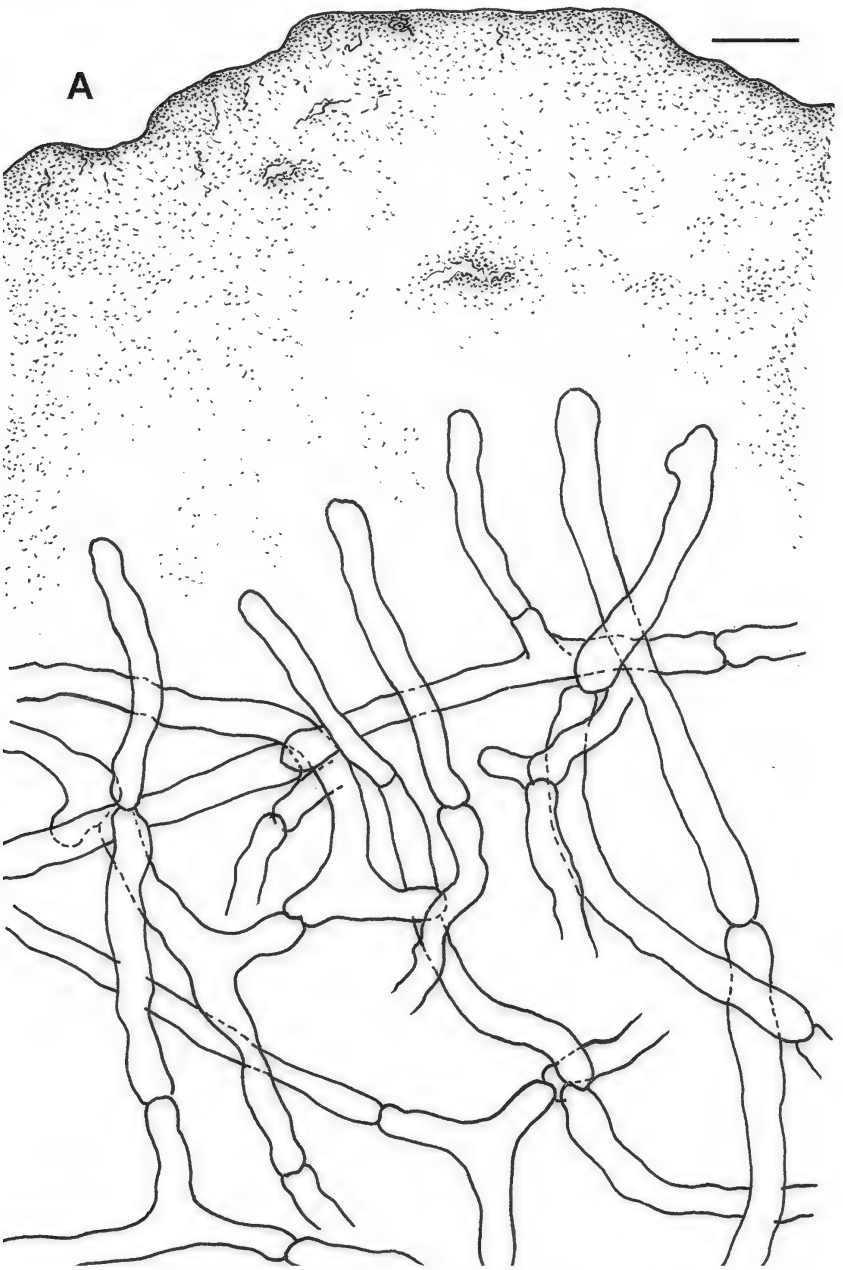

Plate 9. *Lactarius pyriodorus* (Holotype KD 11-027):.
A. Radial section through pileipellis. Scale bar = 10 µm.

Additional specimens examined: **INDIA**. **Sikkim**: North District, Dombang, 27°44′08.7″N 88°45′58.3″E, alt. 2975 m, 14.VIII.2009, A. Verbeken, K. Das & K.V. Putte, AV-KD-KVP 050 & 051 (BSHC, GENT); alt. 2945 m, 23.VIII.2011, K. Das, KD 11-068 (BSHC); North District, Zema, 27°47′20.0″N 88°32′56.1″E, alt. 3079 m, 31.VIII.2012, K. Das, KD 12-223, KD 12-226 (BSHC).

Notes — The viscid to glutinous subzonate pileus, ixocutis to ixotrichoderm nature of the pileipellis, and latex that stains the context and lamellae distinctly lilac to violet place this species in *L.* sect. *Uvidi*. Only one representative of *L.* subg. *Piperites* with lilac milk has been described from Asia (northern Thailand)—*L. formosus* H.T. Le & Verbeken (Le et al. 2007). *Lactarius formosus* clearly differs from *L. pyriodorus* because its pileus is completely covered with bundles of glutinous hairs.

In the ITS-generated phylogenetic tree, the resolution in the group of lilac staining species is very low, and the Indian species is not distinctly separated from a cluster with the European species *Lactarius luridus* (Pers.) Gray and *L. brunneoviolaceus* M.P. Christ. We have previously observed that in this group the morphological variation appears much higher than the ITS molecular variation. Since we observe clear morphological differences between *L. pyriodorus* and the European taxa (also not supported in this tree, although generally accepted as separate species), we choose to present these Indian specimens as a new species. Its darker colours and subzonate aspect are shared with *L. luridus*, which typically associates with broadleaf trees and can be separated by its pileus that is spotted but never with thick gluten (as in *L. pyriodorus*). Also, in *L. luridus,* the stipe base is never strigose and the distinctive sweetish pear-like odour is absent (Heilmann-Clausen et al. 1998). *Lactarius brunneoviolaceus* and *L. pseudouvidus* Kühner are two arctic-alpine species associated with *Salix* that differ greatly from our Indian species in their very small habit. *Lactarius brunneoviolaceus* is further distinguished by its cedar-oil odour and rather large spores (8.8–11.9 × 6.7–8.6 µm) (Heilmann-Clausen et al. 1998, Basso 1999). *Lactarius pseudouvidus* differs microscopically by its distinctly lower (≤0.3 µm) spore ornamentation and differently shaped hymenial cystidia (cylindric to subfusiform with rounded apex) (Heilmann-Clausen et al. 1998). *Lactarius pyriodorus* is superficially similar to the European *L. uvidus* (Fr.) Fr., which can also occur under coniferous trees, but it never has such dark colours (Heilmann-Clausen et al. 1998), and its spores have rounded warts and ridges forming a more incomplete reticulum.

This is for the first time that distinct sweetish odour reminiscent of pears (also known from the European *Inocybe corydalina*) has been encountered in *Lactarius*.

Lactarius yumthangensis K. Das & Verbeken, **sp. nov.** PLATE 10–12, 13C,D,F

MYCOBANK MB 804890

Differs from *Lactarius trivialis* by its lighter spore print, smaller spores, and pileipellis ixocutis.

TYPE: India. Sikkim: North District, Yumthang valley of Shingba Rhododendron sanctuary, 27°46′51.9″N 88°42′37.6″E, alt. 3586 m, 30.VIII.2011, K. Das, KD 11-147 (**Holotype**, BSHC, **isotype**, GENT).

ETYMOLOGY: after the name of the locality from where the type specimen was collected

PILEUS 37–98 mm diam., at first convex with inrolled margin, becoming planoconvex to applanate with depressed indented centre or funnel shaped at maturity, surface smooth, viscid when dry, slimy when moist, vinaceous grey (a: 80) or slightly paler (purplish grey), mostly with distinct darker spots forming zonations; margin non-striate, paler, decurved with maturity. LAMELLAE broadly adnate to decurrent, close to medium crowded (9–11/cm at margin), some forked, with lamellulae in 5 series, cream-yellow to yellow (a: 6F) or ochraceous, sometimes with rusty spots after maturity, unchanging when bruised; edge entire, concolorous. STIPE 21–50 × 9–22 mm, cylindrical to ventricose, often constricted below the juncture of the lamellae, surface slightly longitudinally venose, whitish on constriction, then vinaceous to vinaceous grey (a: 80), cream yellow towards base, with ochraceous areas/spots towards base after maturity. CONTEXT yellowish white to cream, hollow, multi-chambered in stipe, becoming greenish with $FeSO_4$ and orange-yellow with KOH. LATEX chalky white, changing to orange-yellow with KOH, turning pale cream (a: 3C) after some time, slowly (on drying) greenish yellow (a: 57) after 2 hours (on cut lamellae). TASTE acrid. ODOUR fruity. SPORE PRINT pale yellow (c: 30Y, 2M).

BASIDIOSPORES 6.4–**7.1**–8.0 × 5.3–**6.0**–6.9 µm, (Q = 1.06–**1.18**–1.32), subglobose to ellipsoid; ornamentation amyloid, ≤1 µm high, composed of rather regular, narrow and acute ridges mostly arranged in parallel groups forming zebroid pattern, short ridges and isolated irregular warts present between ridges, but, never forming reticulum; plage amyloid distally but often not distinguishable. BASIDIA 38–58 × 9–11 µm, 2–4-spored, clavate to ventricose; sterigmata long, 5–9 × 2–2.5 µm. PLEUROMACROCYSTIDIA 44–90 × 7.5–10 µm, abundant, clavate to fusiform (sometimes with mucronate apex), emergent ≤36 µm; content slightly dense but never needle-like, slightly thick-walled (wall up to 0.6 µm). PLEUROPSEUDOCYSTIDIA irregularly tortuous with rounded apex, 2.5–4 µm wide; contents dense. LAMELLAR EDGE sterile. CHEILOMACROCYSTIDIA 27–55 × 8–9 µm, subclavate or narrowly clavate to fusoid, emergent ≤25 µm, slightly thick walled (wall ≤0.7 µm). MARGINAL CELLS forming chains of elements, terminal cells 11–18 × 5–8 µm, subcylindric to subclavate or clavate, hyaline. SUBHYMENIUM ≤28 µm thick, cellular.

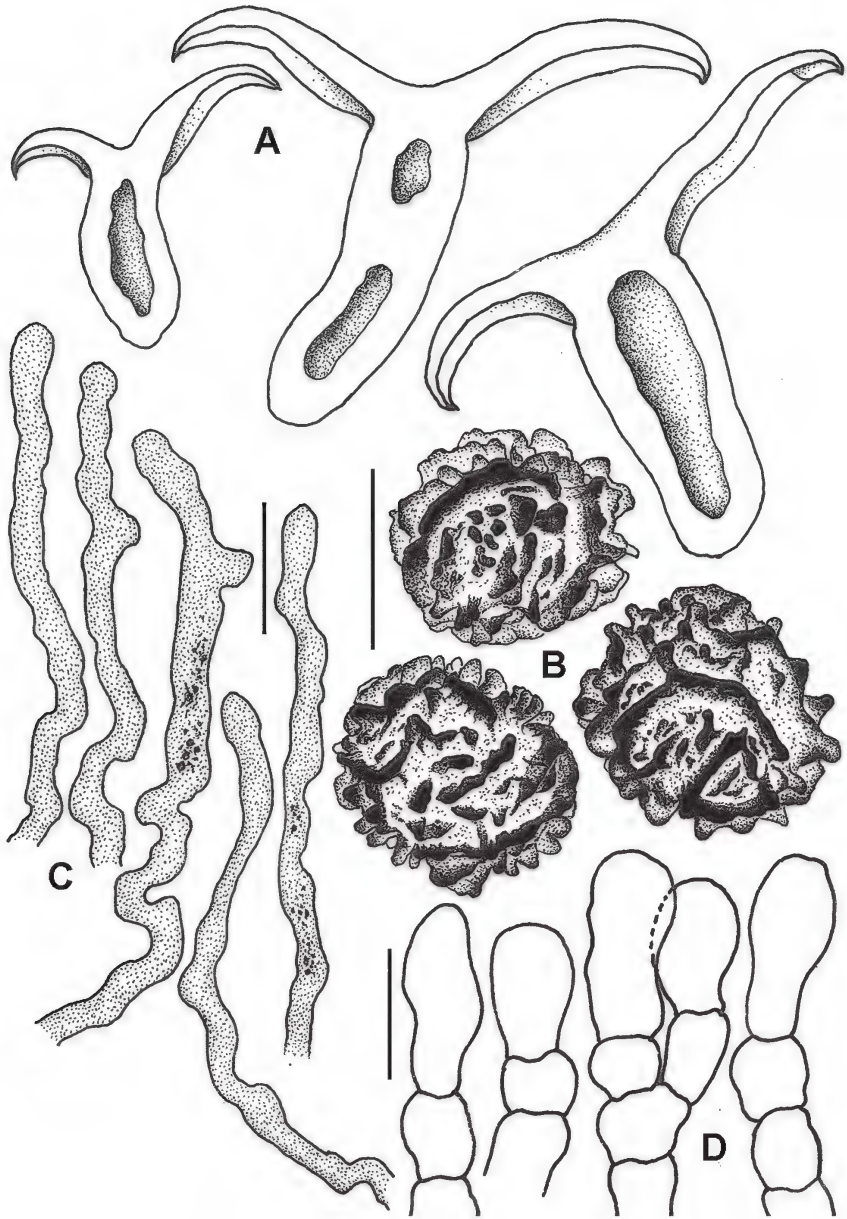

Plate 10:. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Fresh basidiomata showing lamellae and lamellulae. B. Basidiospores.
C. Pleuropseudocystidia. D. Marginal cells. Scale bars: B = 5 µm; C, D = 10 µm.

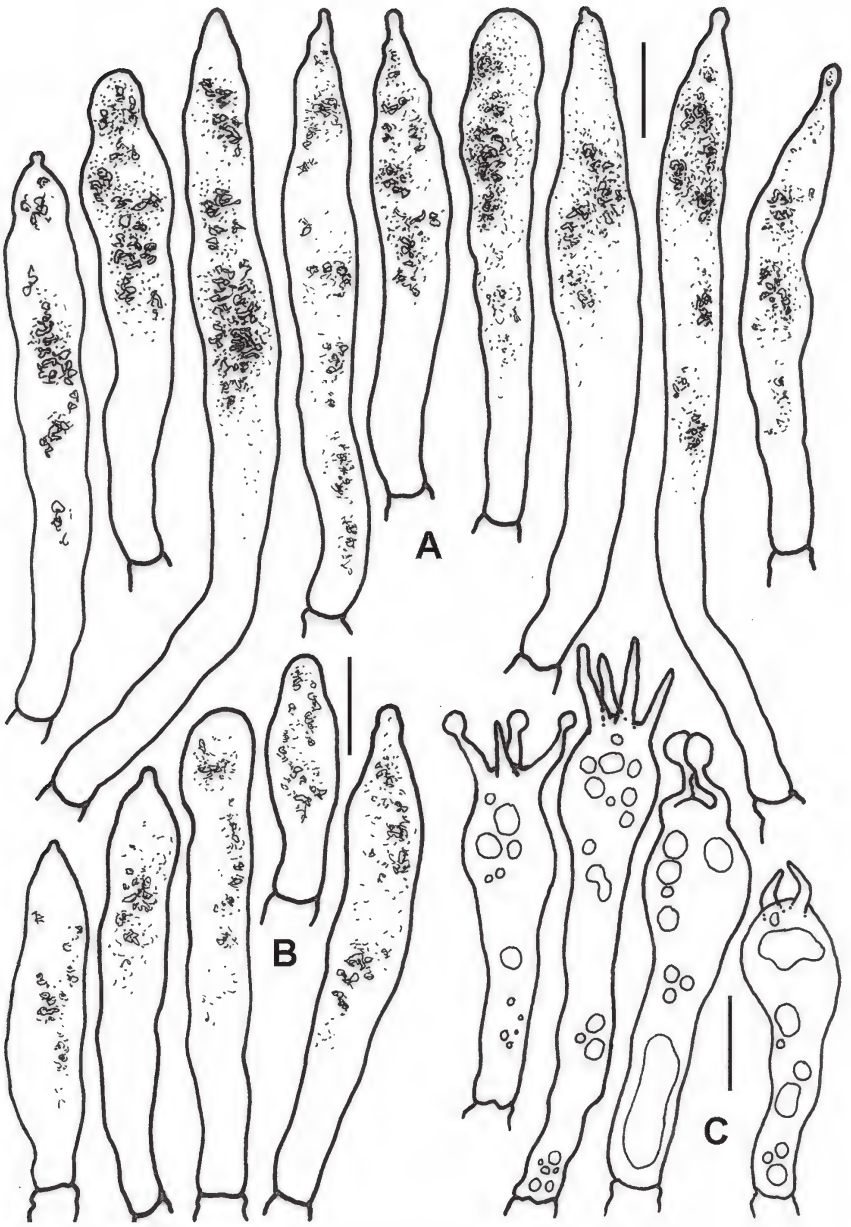

Plate 11. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Pleuromacrocystidia. B. Cheilomacrocystidia. C. Basidia. Scale bars = 10 µm.

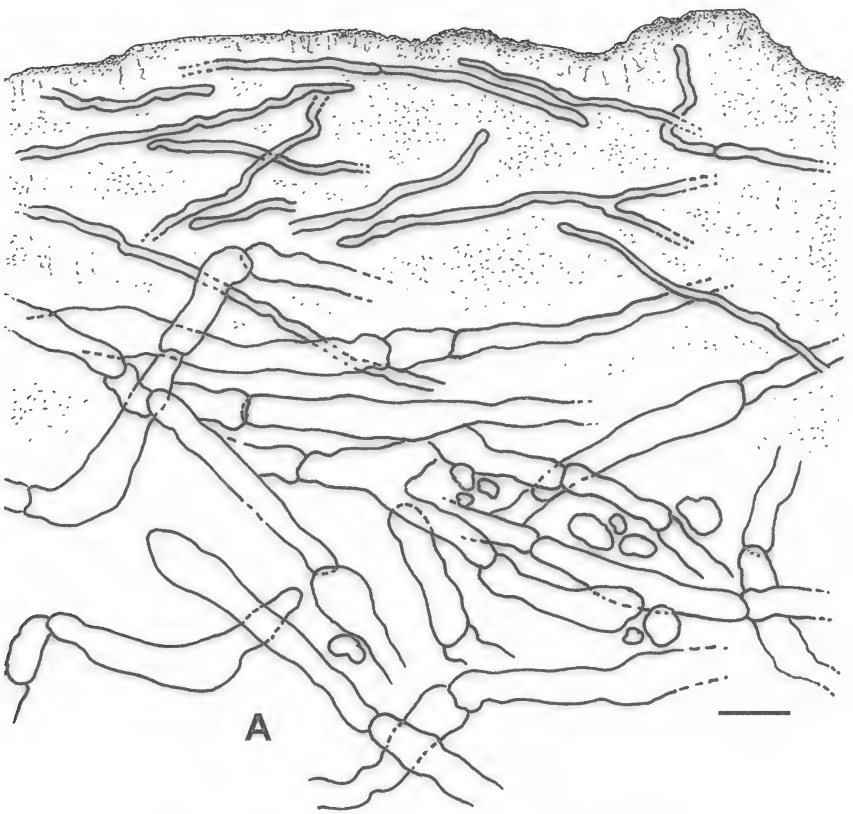

Plate 12. *Lactarius yumthangensis* (Holotype KD 11-147).
A. Radial section through pileipellis. Scale bar = 10 µm.

Pileipellis an ixocutis, composed of narrow repent hyaline hyphae and broad (≤7 µm) septate hyphae embedded in a layer of slime. Clamp connections absent.

Ecology & Distribution — Gregarious to caespitose under *Betula utilis* in subalpine mixed (coniferous and broadleaf) forest. August–September. Uncommon.

Additional specimens examined: **INDIA**. **Sikkim**: North District, Yumthang valley of Shingba Rhododendron sanctuary, 27°46′51.9″N 88°42′37.6″E, alt. 3586 m, 29.VIII.2011, K. Das, KD 11-150 (BSHC); 27°45′11.1″N 88°43′47.5″E, alt. 3580 m, 01.IX.2011, K. Das, KD 11-172 (BSHC).

Notes —The viscid to slimy zonate pileus and the ixocutis to ixotrichoderm nature of the pileipellis undoubtedly place the present species in *L.* subg.

Plate 13. ***Lactarius pyriodorus***. A. Fresh basidiomata. B. Latex oozes out from cut lamellae. E. Basidiospores (SEM). ***Lactarius yumthangensis***. C. Fresh basidiomata. D. Basidiomata showing constricted stipe-apex. F. Basidiospores (SEM). Scale bars: E = 5 µm; F = 3 µm.

Piperites. *Lactarius yumthangensis* can be recognized by its vinaceous grey to purplish grey pileus, venose stipe with a constricted apex, chalky white latex

that changes to orange-yellow with KOH and becomes greenish yellow after long exposure, and growth under *Betula* sp.

Morphologically, *L. flexuosus* (Pers.) Gray and *L. trivialis* (Fr.) Fr. (both reported from Europe) appear quite similar. However, both can be distinguished from *L. yumthangensis* by the slightly darker spore print (c: 40Y, 5M). Moreover, the white latex of *L. flexuosus* is unchanging (never becoming greenish yellow after exposure), the stipe is not constricted at the apex (Kränzlin 2005), and some cheilomacrocystidia have a moniliform apex (Heilmann-Clausen et al. 1998). In *L. trivialis*, spores are distinctly larger (7.3–10 × 5.9–7.8 µm) and the pileipellis is an ixocutis to ixotrichoderm (Heilmann-Clausen et al. 1998).

Acknowledgments

The authors are thankful to the Director, Botanical Survey of India, Kolkata (India) and Department of Forest, Environment and Wild Life Management, Government of Sikkim, for providing all kinds of facilities during this study. They are grateful to Prof. N.S. Atri (India) and Dr. Xiang-Hua Wang (China) for reviewing the manuscript and giving valuable suggestions. Thanks are also due to Ursula Eberhardt, who kindly provided the sequences for the specimens. Assistance (during macrofungal surveys to Dombang, Shingba Rhododendron sanctuary, and Zema) rendered by S.K. Rai, S. Pradhan, R. Giri, R.K. Ram and P. Tamang (BSI, SHRC, Gangtok) is duly acknowledged.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

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http://dx.doi.org/10.5248/130.131

Volume 130, pp. 131–143 *January–March 2015*

Synonymy of two species of *Bipolaris* from aquatic crops of *Poaceae*

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Abstract— Our morphological data indicate that conidia of *Bipolaris zizaniae* (a pathogen causing brown spot on leaves of *Zizania latifolia*) are similar to those of *B. oryzae*. Our sequences of the rDNA ITS, GPDH, and EF1-α gene regions from *B. zizaniae* and blast searches revealed a 99–100% similarity with sequences of *B. oryzae*. Phylogenetic studies also cluster *B. zizaniae* isolates with *B. oryzae* in a clade with 100% bootstrap support. Pathogenicity testing also confirmed that *B. zizaniae* does infect both *Zizania latifolia* and rice, causing brown spots.

Key words—graminicolous fungi, molecular phylogeny, taxonomy

Introduction

Zizania latifolia (Griseb). Turcz. ex Stapf is a perennial grass parasitized by the smut fungus *Ustilago esculenta* Henn., which causes swelling of its upper culms. The swollen culms are used as an aquatic vegetable, commonly called jiaobai (white bamboo) in China (Zhang et al. 2012). Due to its unique flavor and delicacy, the demand for jiaobai has steadily increased in tandem with the rapid economic development of China. Zhejiang Province has become the largest *Z. latifolia* cultivation area in China (Xu et al. 2009). Brown spot is one of the most severe leaf spot diseases of this crop plant. With large-scale cultivation and changed growing conditions, brown spot incidence has become more severe in the fields, and the disease has become a severe problem in crop production. The pathogen causes a large number and areas of coalescing spots (especially during seedling production in greenhouses), and these spots expand or coalesce to form large lesions, eventually resulting withering and death of entire leaves (Fig. 1A–D).

The pathogen causing this disease was first described as *Helminthosporium zizaniae* by Nisikado (1929). Although *H. zizaniae* resembles *H. oryzae* morphologically and by infecting *Oryza sativa* L. [rice], Nisikado (1929) differentiated the two species based on the shape of the conidial base, conidial dimensions, and conidial hilum. Shoemaker (1959) transferred *H. zizaniae* and *H. oryzae* to *Bipolaris,* a placement later accepted by Shoemaker (1966) and Sivanesan (1987). Sivanesan (1987) used conidial dimensions as an important character in his dichotomous key to *Bipolaris;* he distinguished *B. zizaniae* as having >150 µm long conidia compared with the <150 µm long conidia in *B. oryzae*. However, conidial dimensions are usually variable in *Bipolaris* (Sivanesan et al. 1987; Manamgoda et al. 2012), and the conidial length in *H. oryzae* is not stable and can be more than 150 µm in some strains (Chang 1978). Other studies showed that *B. oryzae* is interfertile with *B. zizaniae* and that their isolates from rice and *Zizania* are reciprocally infective (Chang 1974; Tsuda & Ueyama 1975, 1976). *Bipolaris zizaniae* and *B. oryzae* are now regarded as conspecific (Manamgoda et al. 2014).

Accurate identification and precise naming of species are crucial, for species names provide an ideal framework for storage and information retrieval for effective disease management (Inderbitzin et al. 2011, Manamgoda et al. 2012). *Bipolaris zizaniae* is an important pathogen on *Z. latifolia*, but due to limited distribution, there is little information on its effects; even the morphological descriptions of the pathogen are inadequate (Nisikado 1929, Sivanesan 1987). In contrast, *B. oryzae* [sexual state previously known as *Cochliobolus miyabeanus*] causes brown spots on leaves and glumes of rice, leading to serious losses in yield (Scheffer 1997). Thus this pathogen and rice disease have been studied worldwide (Kulkarni et al. 1980, Kubo et al. 1989, Amadioha 2002, Manandhar et al. 1998, Xio et al. 1991). Nonetheless, the relationship between *B. zizaniae* and *B. oryzae* is unresolved. With the use of molecular taxonomy, DNA sequence analyses allow testing the morphological species circumscriptions and providing new insights into species relationships, especially for complexes of closely related, morphologically similar species (Manamgoda et al. 2012, 2014; Udayanga et al. 2012; Hyde et al. 2014). Studies of *Bipolaris* species have already revealed relationships between morphological species circumscriptions and molecular data, but there has been no study of *B. zizaniae* (Manamgoda et al. 2011, 2012, 2014; Hyde et al. 2014).

We used sequences from the ITS (internal transcribed spacer), GPDH (glyceraldehyde-3-phosphate dehydrogenase), and EF1-α (translation elongation factor 1-α) gene regions to investigate the phylogenetic relationships of six *Bipolaris* cultures isolated from *Zizania latifolia* in southeast China. The objectives were to review morphological similarities in *B. zizaniae* and *B. oryzae* and to resolve their relationship.

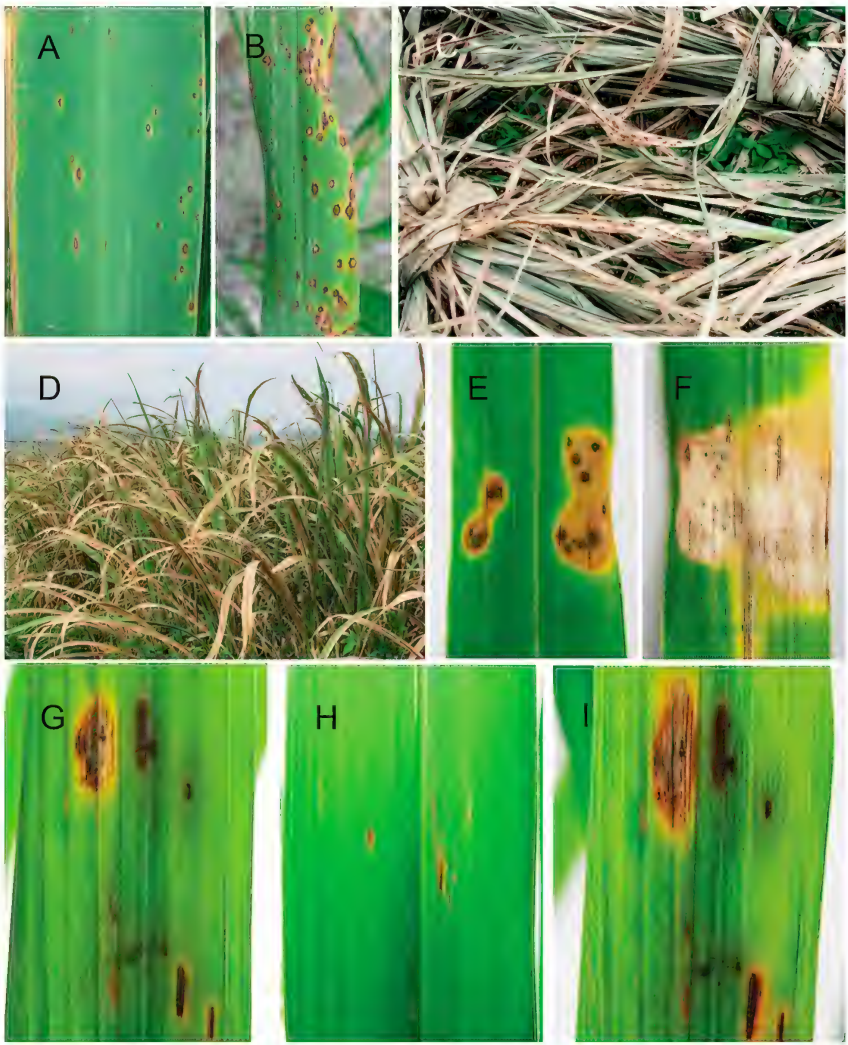

FIGURE 1 Symptoms of *Bipolaris* brown spot on *Zizania latifolia* and *Oryza sativa*. A–D. On nature host of *Z. latifolia*. A. Early spots. B. Severe spots. C. Removed leaves with severe brown spots from seedlings in the greenhouse. D. Leaf blight. E–F. Symptom variability on backs of leaves of *Z. latifolia* after artificial inoculation. E. Five days after inoculation. F. Eighteen days after inoculation. G–I. Symptom variability on leaves of *O. sativa* after artificial inoculation. G. Brown spots eight days after inoculation. H. Brown spots on rear of leaf nine days after inoculation. I. Brown spots twelve days after inoculation.

TABLE 1. *Bipolaris* and *Curvularia* isolates used in multi-gene DNA sequence analysis.

SPECIES	ISOLATE	HOST	LOCATION	GENBANK NO.		
				ITS	GPDH	EF1-α
B. coffeana	ICMP 6128	*Cynodon dactylon*	New Zealand	JX256412	JX276427	JX266581
B. microlaenae	CBS 280.91 *	*Microlaena stipoides*	Australia	JN601032	JN600974	JN601017
	BRIP 15613			JN192378	JN600973	JN601017
B. nodulosa	CBS 160.58			JN601033	JN600975	JN601019
B. oryzae	MFLUCC 10-0715 *	*Oryza sativa*	Thailand	JX256416	JX276430	JX266585
	MFLUCC 10-0694	*O. sativa*	Thailand	JX256413	JX276428	JX266582
	MFLUCC 10-0733	*O. sativa*	Thailand	JX256417	JX276431	JX266586
	MFLUCC 13-0511	***Zizania latifolia***	**China**	**KF688965**	**KF688971**	**KF688977**
	MFLUCC 13-0512	***Z. latifolia***	**China**	**KF688966**	**KF688972**	**KF688978**
	MFLUCC 13-0513	***Z. latifolia***	**China**	**KF688967**	**KF688973**	**KF688979**
	MFLUCC 13-0514	***Z. latifolia***	**China**	**KF688968**	**KF688974**	**KF688980**
	MFLUCC 13-0515	***Z. latifolia***	**China**	**KF688969**	**KF688975**	**KF688981**
	MFLUCC 13-0516	***Z. latifolia***	**China**	**KF688970**	**KF688976**	**KF688982**
B. peregianensis	BRIP 12790 *	*Cynodon dactylon*	Australia	JN601034	JN600977	JN601022
C. australiensis	CBS 172.57	*Oryza sativa*	Vietnam	JN601026	JN601036	JN601003
C. coicis	CBS 192.29 *	*Coix lacryma*	Japan	JN192373	JN600962	JN601006
C. ellisii	CBS 193.62		Pakistan	JN192375	JN600963	JN601007
C. graminicola	BRIP 23186a		Australia	JN192376	JN600964	JN601008
C. hawaiiensis	CBS 173.57 *	*Oryza sativa*	Hawaii, USA	JN601029	JN600966	JN601010
	BRIP 1097	*Chloris gayana*	Australia	JN601030	JN600967	JN601011
C. heteropogonis	CBS 284.91 *	*Heteropogon contortus*	Australia	JN192379	JN600969	JN601013
C. homomorpha	CBS 156.60			JN192380	JN600970	JN601014
C. lunata	CBS 730.96 *		USA	JX256429	JX276441	JX266596
	MFLUCC 10-0706	*Oryza sativa*	Thailand	JX256431	JX276443	JX266598
C. ovariicola	BRIP 15882			JN601031	JN600976	JN601015
C. ravenelii	BRIP 13165	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
C. spicifera	CBS 274.52		Spain	JN192387	JN600979	JN601023
C. tripogonis	BRIP 12375		Australia	JN192388	JN600980	JN601025
C. tuberculata	CBS 146.63 *	*Zea mays*	India	JX256433	JX276445	JX266599

* = ex-type cultures. New sequences are presented in bold font; other sequences are from Manamgoda et al. 2011, 2012, 2014.

Materials & methods

Collection and isolation of fungi

Diseased leaves of *Zizania latifolia* with brown spots growing in fields or greenhouses were collected and returned to the laboratory. Tissue pieces (each approximately 5 × 5 mm) were cut from the margins of brown spots, between healthy and infected parts of leaves, which were previously surface disinfected with 0.5% NaOCl. The pieces were plated on to PDA medium and incubated at 25°C for 7 days. Mycelia from the infected samples were transferred to fresh PDA medium. Single-spore isolation was performed as described by Chomnunti et al. (2014). Agar discs (with mycelium on the surface, each approximately 5 × 5 mm) from these isolates were stored in 1.5 mL cryotubes with 20% glycerol at –70°C.

Herbarium materials are deposited at MFLU herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The cultures are maintained at Mae Fah Luang University Culture Collection (MFLUCC) and Institute of Biotechnology, Zhejiang University, Zhejiang Province, China (ZJU).

DNA extraction

Genomic DNA was extracted using a modified protocol of Zhang and Li (2009). Fungal isolates were cultured on PDA at 25°C for 7 days. Fresh mycelia were scraped from the surfaces of PDA plate and grounded to a fine powder in liquid nitrogen. 500 mg power was transferred to a 1.5 mL microcentrifuge tube and 700 µL of preheated (60°C) 2× CTAB extraction buffer (2% hexadecyltri-methlammonium bromide(w/v), 100 mM Tris–HCl, 1.4 M NaCl, 30 mM EDTA, pH 8.0) was added. The solution was incubated in a water bath at 65°C for 60 min with a gentle swirling. Following a phenol/chloroform extraction, the genomic DNA was precipitated by isopropanol in the presence of sodium acetate. The precipitate was rinsed with 70% ethanol centrifuged at 12,000 rpm and genomic DNA was resuspended in 50 µL TE buffer and stored in –20°C.

PCR amplification

The DNA fragments were amplified in an automated thermal cycler (Eppendorf AG, Germany). Amplification was performed in a 50 µL reaction volume which contained 5 µL 10× PCR buffer, 1 µL of each primer (10 µM), 2 µL template DNA, and 0.5 µL Taq DNA polymerase. Primers ITS6 and ITS4 (Zhang & Li. 2009) were used to amplify the ITS and 5.8 S regions. The thermal cycling program was performed with 35 cycles after an initial denaturation at 95°C for 4 min. Each cycle included a denaturation step at 95°C for 1min, an annealing step at 55°C for 1min, and an extension step at 72°C for 1.5 min. The primers gpd1/ gpd2 (Berbee et al. 1999) were used for amplifying the GPDH gene with the changes of annealing temperature 52°C. Similarly, the EF-1α gene was amplified using primers EF 983/2218R (Schoch et al. 2009) with the changes of annealing temperature 54°C.

Sequence alignment and phylogenetic analysis

The purified PCR products were submitted to Sunny Biotechnology Company Limited (Shanghai, China) for sequencing in both directions. Sequence files were assembled and edited using BioEdit software (Hall 1999). A blast search was performed

for searching the highly similar sequences in the GenBank database and related sequences were downloaded (Table 1). The sequences were aligned with Clustal X 25 (Thompson et al. 1997). Sequences were analyzed phylogenetically in PAUP v4.0b10 (Swofford 2002) and trees were visualized with TreeView (Page 1996). Phylogenetic trees were inferred from the ITS, GDPH and EF1-α sequence data set using parsimony analysis with all characters weighted equally and all gaps treated as missing data.

Morphological studies

Conidia and conidiophores taken directly from nature hosts were examined in 3% KOH, using a Zeiss Axiophot 2 microscopy with Axiocam CCD camera and AxioVision digital imaging software (AxioVision Software Release 3.1.v.3–2002; Carl Zeiss Vision Imaging Systems). Cultural characteristics and morphology were determined on PDA, and water agar and wheat straw (WSA) [2 % water agar (10 g/L) with autoclaved wheat straw placed onto the medium] and incubated for 7–10 days at 25°C under 12/12 h alternation of near-UV light. 20 µL conidial suspension (10^4 conidia/mL) were dropped on the WA (water agar) medium at 25°C for 24 h and characteristics of conidia germination were observed.

Scanning electron microscopy

The 0.5 × 0.5 mm^2 pieces from diseased spots with conidia and conidiophores were placed in glutaraldehyde (2.5% vol/vol) for 48 h at 4°C. Samples were washed three times in 0.1 M sodium phosphate buffer (pH 7.0) and post-fixed in osmium tetroxide (1% wt/vol) for 1.5 h at 20°C. Samples were rinsed thoroughly with 0.2 M phosphate buffer (pH 6.8) and dehydrated in a graded ethanol series (30, 50, 70, 80, 90, 95, and 100%). Pieces were embedded in Spurr's epoxy resin. The sites containing conidia were identified by light microscopy and ultrathin sections were cut with a Reichert-Jung Ultracut E Ultramicrotome with a diamond knife. Ultrathin sections were collected on Formvar-coated slot copper grids; after drying, the grids were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope (Hitachi S-3000N, Tokyo, Japan).

Pathogenicity Tests

Healthy field cuttings of *Z. latifolia* with 3–5 nodes were cultivated in a 25-cm diam. barrel with cultivated soil and placed in greenhouse at 26–28°C for one month. *Oryza sativa* seedlings grown in seedbeds were transplanted into a 20-cm diam. barrel and placed in greenhouse under the same conditions for one month.

An aqueous inoculation suspension (1 × 10^5 conidia/mL) with 1% vol/vol Tween-20 was prepared from 8–10-day old cultures. Pathogenicity tests were conducted on healthy leaves of *Zizania latifolia* and *Oryza sativa*. A 50 µL conidial suspension was dropped on two sides of each leaf and then inoculated plants were covered by large plastic bags and incubated at 27–28°C for 48 h after which the plastic bags were removed. Control leaves were inoculated with 50 µL sterile water. Symptoms and sizes of diseased spots were observed and recorded each day. The fungus was re-isolated by cutting small portions from the margin of diseased spots; these were surface sterilized and placed on PDA plates. The experiments were repeated twice to confirm the results.

Results

Phylogenetic analysis

Isolates of *Bipolaris* were obtained from *Z. latifolia* and purified, and six single-spore isolates were selected for study. Six strains of *Bipolaris* from *Z. latifolia* had identical ITS and GPDH gene sequences, but in the EF-1a gene sequence there was one base difference between the two strains from Hangzhou (MFLUCC13-0511, MFLUCC13-0512) and the other four strains (MFLUCC13-0513, MFLUCC13-0514, MFLUCC13-0515, MFLUCC13-0516). A GenBank search showed that there were no sequence data submitted for *B. zizaniae,* but a blast search of our ITS, GPDH, and EF1-α sequences from

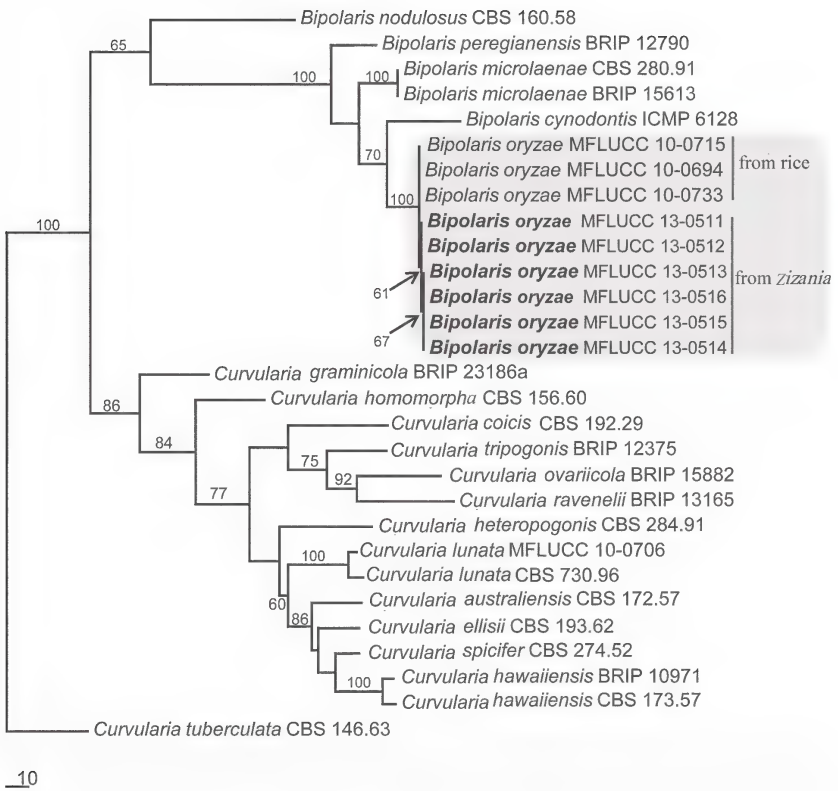

FIGURE 2. Phylogram generated from parsimony analysis based on ITS, GPDH and EF 1-α sequence data of *Bipolaris* and *Curvularia* species. Data were analysed with random sequence addition, unweighted parsimony and by treating gaps as missing data. Bootstrap values ≥50% are indicated. Species names are followed by the number of the deposited voucher. The tree is rooted with *Curvularia tuberculata.*

B. zizaniae showed a 99–100% similarity with those of *B. oryzae* lodged in GenBank. Of the 63 reliable or voucher *B. oryzae* ITS sequences in GenBank, 11 were identical with *B. zizaniae* and 41 differed by 1–2 bases. Similarly, of the 16 reliable or voucher *B. oryzae* GPDH sequences in GenBank, one was identical with *B. zizaniae* and 15 differed by 1–2 bases. Finally, of five *B. oryzae* EF1-α sequences (Manamgoda et al. 2012), four were identical with *B. zizaniae* and one differed by 1–2 bases.

A parsimony tree was constructed from the ITS, GPDH, and EF1-α data from 29 fungal isolates (Table 1). The four *Bipolaris* isolates from *Z. latifolia* with identical sequences grouped together with another two *Bipolaris* isolates from *Z. latifolia* (with one base difference) with 61% bootstrap value support (Fig. 2). This group clustered with three *B. oryzae* isolates with a 100% bootstrap support. Fig. 2 clearly shows that the *Bipolaris* isolates from *Z. latifolia* are inseparable from *B. oryzae*. Sequence variation between *Bipolaris* isolates from *Z. latifolia* and *B. oryzae* isolates was less than interspecific variation. Six *Bipolaris* isolates from *Z. latifolia* and three *B. oryzae* isolates clustered with *B. coffeana* Sivan. to form a clade with a 70% bootstrap value support (Fig. 2). However, the *B. coffeana* subclade (based on a single culture) and the *B. oryzae*/*B. zizaniae* subclade were separated by >40 base differences, indicating that they are not conspecific.

The description below is based on our collections of *Bipolaris* isolates from *Z. latifolia*. Except for size variation, these characters are almost identical to those from the type specimen from the same host from Japan (Nisikado 1929). The pathogen causes brown spots on leaves of *Zizania latifolia* and infects almost all aerial parts of the plant.

Bipolaris oryzae (Breda de Haan) Shoemaker, Can. J. Bot. 37: 883 (1959) Fig. 3

≡ *Helminthosporium oryzae* Breda de Haan, Bulletin Inst. Bot. Buitenzorg 6: 11 (1900)

= *Ophiobolus miyabeanus* S. Ito & Kurib., Ann. Phytopath. Soc. Japan 2(1): 7 (1927)

≡ *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur, Indian. J. Agr.Res. 12: 733 (1942)

= *Helminthosporium zizaniae* Y. Nisik., Ber. Ohara Inst. Landw. Forsch. Kurashiki 4: 122 (1929)

≡ *Bipolaris zizaniae* (Y. Nisik.) Shoemaker, Can. J. Bot. 37: 885 (1959)

Leaf spots dark brown with a paler centre 0.3–0.5 × 0.3–0.6 cm. On a natural host, conidiophores single or occasionally fasciculate in small groups, rarely branched, geniculate or straight in sterile part, then becoming slightly to distinctly geniculate, cicatrized with scars often inflated at base, medium olivaceous-brown below, paler at the apex, multiseptate, 106–395 μm long, swollen to 7–9 μm diam at base, then narrowing to 6–7 μm diam (middle)

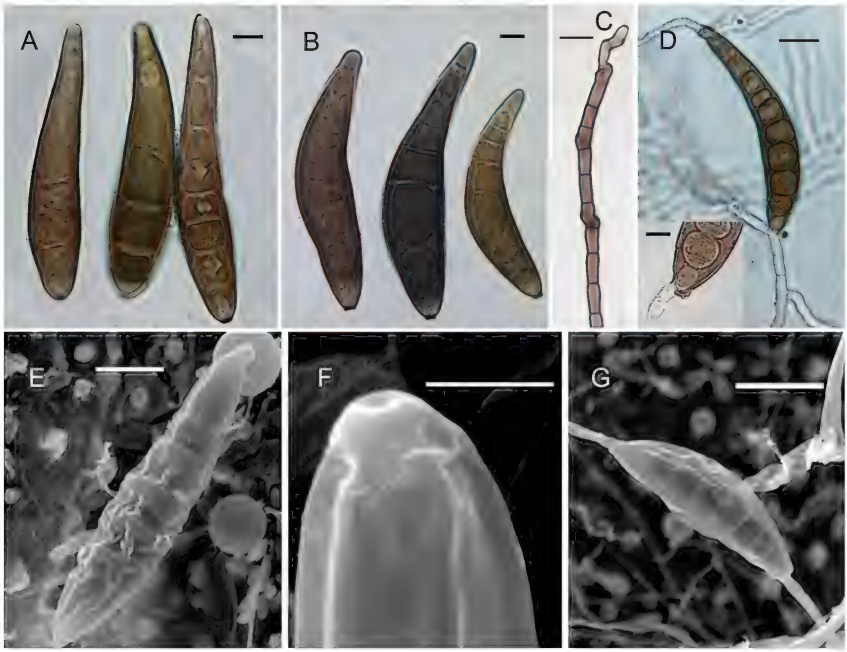

FIGURE 3. *Bipolaris oryzae* (isolates from Z. *latifolia*). A–D. Light microscope micrographs. A. Conidia. B. Conidia on WSA. C. Conidiophore. D. Germinated conidium with one germ tube at each end. Basal germ tube direction of growth (left below) is semiaxial, close to hilum. E–G. SEM micrographs. E. Conidia. F. Hilum. G. Germinated conidium with two polar germ tubes. Scale bars: A, B, D(lower) = 10 μm; C, D(upper) = 20 μm; E = 15 μm; F = 5 μm; G = 25 μm.

and 3–5 μm (apex). Conidia (65–)78–110(–131) × (15–)17–28(–34) μm, 6–10 distoseptate, olivaceous brown or yellowish brown, obclavate, navicular, straight or slightly curved, tapering towards the apex, hilum dark, conspicuous, sometimes slightly protruding. Conidia germinate by the production of two polar germ tubes. The sexual morph is not found in nature.

On WSA, conidiophores 212–646 × 5.5–10.5 μm, olivaceous-brown, paler at the apex. Conidia brown or dark brown, obclavate, fusoid, often curved, (73–)80–125(–130) × (1–)18–28(–31) μm, 6–11-distoseptate, hilum dark, conspicuously protruding or truncate.

MATERIALS EXAMINED: **CHINA**, **ZHEJIANG PROVINCE**, Hangzhou Suburbs, September 2012, Zilan Xiao (MFLU13-0092; cultures MFLUCC13-0511 = ZJU0101, MFLUCC13-0512 = ZJU0102); **ANHUI PROVINCE**, Yuexi County, September 2012, Zilan Xiao (MFLU13-0093; cultures MFLUCC13-0513 = ZJU0201, MFLUCC13-0514 = ZJU0202); **FUJIAN PROVINCE**, Putian city, October 2012, Zilan Xiao (MFLU13-0094; cultures MFLUCC13-0515 = ZJU0301, MFLUCC13-0516 = ZJU0302).

Table 2. Synopsis of conidial size and number of distosepta in *Bipolaris oryzae* from *Zizania latifolia* from different origins.

	Isolate	Length (μm)	Width (μm)	Distosepta
On hosts	MFLU 13-0092	99 ± 11.5	25 ± 3.0	(5–)7–9
	MFLU 13-0093	87 ± 8.7	20 ± 3.6	(5–)7–10
	MFLU 13-0094	92 ± 10.6	23 ± 3.5	(5–)6–10
On WSA	MFLUCC 13-0511	96 ± 11	24 ± 3.8	6–9(–11)
	MFLUCC 13-0513	86 ± 7.8	19 ± 5.4	6–10
	MFLUCC 13-0515	90 ± 5.6	21 ± 6.7	6–10(–11)

Comments—Classification in the genus *Bipolaris* was previously based on morphology, with conidial characters being considered as important in species delimitation. In this study conidia of *Bipolaris* isolates from *Z. latifolia* are shorter (78–110 μm) and wider (17–28 μm) than those reported in the protologue (25–166 × 12–13 μm; Nisikado 1929). Although there is some variation in conidial size and distosepta in *Bipolaris* isolates from *Z. latifolia* from different locations in China (Table 2), the morphological differences are not significant. We found it impossible to distinguish *B. oryzae* from *B. zizaniae* based on morphological characters.

Morphology and molecular phylogeny confirm that *Bipolaris zizaniae* and *B. oryzae* are conspecific. *Bipolaris oryzae* is the correct name for this expanded taxon because the epithet *oryzae* (published in 1900) has priority over *zizaniae* (published in 1929). We follow Manamgoda et al. (2012, 2014) in preferring *Bipolaris* to the earlier generic name *Cochliobolus* (proposed for the sexual state).

Pathogenicity testing

The isolates of *Bipolaris* isolated from Z. *latifolia* infected the leaves of *Z. latifolia*. At 48 h after inoculation, *Bipolaris* isolates from *Z. latifolia* induced small flecks or expanded spots on the lower surface of *Z. latifolia* leaves. Symptoms were similar to those observed in the wild (Fig. 1e,f), and no disease was found on upper surface of inoculated or control leaves. Five days after inoculation, diseased spots increased in size (Fig. 1e). Symptoms of leaf blight occurred 18 days after inoculation (Fig. 1f). During the same period, disease symptoms did not develop on the upper surface or on control leaves.

Similarly, isolates of *Bipolaris* isolated from *Z. latifolia* also infected rice leaves. Small flecks occurred on the upper surface of inoculated leaves after five days and similar flecks occurred on the lower surface of leaves after six days. After 8–9 days, diseased spots increased more rapidly in size on the upper surface of leaves than on the lower surface of leaves (Fig. 1g, h). The diseased spots were approximately 0.5 cm in diameter 12 days after inoculation (Fig. 1i).

Discussion

Zizania latifolia, which originated in the northern region of China, was once used as an important grain in ancient times. Cultivated *Z. latifolia* was disseminated to Taihu Lake from north to south, and the Taihu Lake basin is the region associated with the origin of jiaobai (Xu et al. 2008). Jiaobai cultivation was introduced to eastern and southeastern Asia, including the Russian Far East, Japan, and Korea (Thrower & Chan 1980, Guo et al. 2007). It is possible that brown spot was also introduced to the jiaobai-growing areas with the planting material.

Zizania latifolia commonly grows in shallow water and can be found at the edges of lakes, ponds, wetlands, and flooded fields (Thrower & Chan 980, Guo et al. 2007). In some cases, *Z. latifolia* is able to grow in deeper water (Yamasaki & Tange1981), because it has a well-developed ventilation system (Yamasaki 1984, 1987). Thus, *Z. latifolia* growing conditions are similar to that of rice. In the jiaobai-growing areas, *Z. latifolia* is usually grown in paddy fields or in rotation with rice. The fact that pathogenic brown spot of *Z. latifolia* also infects rice may be a result of long-term evolution. In morphological taxonomy, phenotypic plasticity and homoplasy may influence fungal species delimitation (Pino-Bodas et al. 2011). *Bipolaris zizaniae* and *B. oryzae* have previously been delineated based on morphology, although species boundaries are not clear (Chang 1974; Tsuda & Ueyama 1975, 1976). *Bipolaris zizaniae* can be crossed with *B. oryzae* to form the sexual state, and both species can infect *Zizania* (Chang 1974, Tsuda et al. 1975). The data presented here and in previous studies suggest that *B. oryzae* and *B. zizaniae* are synonymous. Our gene sequence data analysis shows unequivocally that *B. oryzae* and *B. zizaniae* are conspecific.

Acknowledgments

The authors are grateful to Dimuthu S. Manamgoda (School of Science, Mae Fah Luang University, Thailand) and Lei Cai (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, People's Republic of China) for their helpful comments on this manuscript. The research was funded by the National Natural Science Foundation of China (No. 31070123) and the Special Fund for Agro-scientific Research in the Public Interest of China (No. 201003004). KD Hyde thanks MFLU grant No. 56101020032 for funds to study *Dothideomycetes.*

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.145

Volume 130, pp. 145–164 *January–March 2015*

Myxomycetes of Sonora (Mexico) 6. Central plains of the Sonoran Desert

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Abstract — Myxomycetes from 10 localities in the central plains of the Sonoran desert were studied using the moist chamber culture technique. Thirty-three species have been identified, of which five (*Comatricha mirabilis, Cribraria confusa, Didymium eremophilum, Perichaena luteola,* and *P. stipitata*) represent new records for Sonora, and *Comatricha mirabilis* is cited for the first time from Mexico. Light and scanning electron microphotographs illustrate the most diagnostic characters of these species, which are rare or scarcely mentioned in the literature.

Key words — *Amoebozoa,* chorology, myxobiota, slime moulds, taxonomy

Introduction

The Sonoran desert, which lies in northwestern Mexico and the southwestern United States, covers a 260,000-km2 area, with the largest portion occurring in Sonora state (Mexico) and the rest in southwestern Arizona and southeastern California (United States). This is the warmest and the most arid North American desert due to its low elevation (≤600 m) and 22–24 °C average annual temperature. The bi-seasonal (winter and summer) rainfall averages 200–400 mm per year (Hernández 2006).

The desert vegetation is characterized by trees and shrubs, including *Parkinsonia florida* (A. Gray) S. Watson, *Olneya tesota* A. Gray, *Prosopis glandulosa* Torr., *Fouquieria macdougalii* Nash, and *Bursera* spp. The majority of the cacti are large, with *Carnegiea gigantea* (Engelm.) Britton & Rose the

Table 1. Studied Sonoran localities and types of vegetation

Locality	Latitude N	Longitude W	Altitude	Vegetation
Town of Benjamin Hill				
1. Las Animas	30°12′37.95″	111°18′55.04″	770 m	MDS
Town of Opodepe				
2. Tuape	30°02′52.89″	111°00′22.05″	670 m	M
Town of Guaymas				
3. Cañón de Nacapule	27°59′08.54″	111°02′06.40″	81 m	SC
4. El Tigre	28°06′31.14″	111°01′21.11″	139 m	SC
5. El Apache	28°19′25.33″	111°14′27.74″	43 m	M
6. Maytorena	28°13′32.49″	110°48′50.28″	40 m	SC
Town of Carbó				
7. San Luis	29°33′42.38″	111°04′52.79″	458 m	MDS
Town of Hermosillo				
8. La Primavera	28°48′06.37″	111°09′09.62″	167 m	MDS
9. La Pintada	28°39′56.35″	110°57′15.64″	233 m	SC
10. El Papalote	29°12′56.14″	111°02′39.43″	348 m	M

Types of vegetation: MDS = microphyllous desert scrub; SC = sarcocaule scrub; M = mezquital

most representative (Hernández 2006). Myxomycetes can develop in arid environments due to decomposition of and water retained by succulent plants such as *Opuntia* spp. These provide an excellent microhabitat with enough moisture for optimal development. Although the Sonoran desert has a high level of biological diversity, here have been only a few studies of its myxobiota.

The Sonoran desert records of Evenson (1961) and Blackwell & Gilbertson (1980a,b; 1984) were obtained mainly in Arizona (U.S.A.) in the United States. Although there have been various diverse studies of the myxobiota in Sonora (Mexico), only twenty-seven reports are known for the biosphere reserve El Pinacate and Gran Desierto de Altar (Esqueda et al. 2013, Moreno et al. 2004); most studies have been centered in pine–oak forests and lowland areas (Pérez-Silva et al. 2001; Moreno et al. 2006; Lizárraga et al. 2007, 2008a,b).

The catalogue of myxomycetes for Sonora state (Moreno et al. 2007) has been increased from 77 species to 108 species by Lizárraga et al. (2007, 2008a,b) and Esqueda et al. (2013). The present contribution brings the total number to 113.

Materials & methods

Four surveys were conducted in 2009, one each season of the year, in 10 localities of the central plains of the Sonoran desert (Table 1). Specimens were gathered or cultured in moist chamber cultures. Permanent slides were prepared in Hoyer's liquid for microscopic study. Collections have been deposited in the collection of fungi of the

Universidad Estatal de Sonora, Hermosillo, Sonora, México (UES), with a small part in the herbarium of the Universidad de Alcalá, Alcalá de Henares, Madrid, Spain (AH). The spore ornamentation was examined under SEM using the material deposited in AH. Collector names are abbreviated as follows: AG = A. Gutiérrez; ML = M. Lizárraga; CS = C. Salazar; and AS = A. Sánchez. New species records for Sonora are marked with a single asterisk, and the species recorded as new for Mexico is marked with two asterisks.

Taxonomy

Arcyria cinerea (Bull.) Pers.

Studied material: **MEXICO. Sonora: Loc. 4**, on *Forchhammeria watsonii* Rose, leg. ML, CS, AS & AG, 19-VIII-2009, obtained from moist chamber, 30-IX-2009 (UES 8539); obtained from moist chamber, 9-X-2009 (UES 8541).

Comments— Previously cited for Sonora by Lizárraga et al. (2007, 2008b).

Arcyria denudata (L.) Wettst.

Studied material: **MEXICO. Sonora: Loc. 1**, on decomposing wood, leg. AG & AS, 22-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8106).

Comments—Previously recorded for Sonora by Pérez-Silva et al. (2001), and Lizárraga et al. (2008a,b).

Badhamia gracilis (T. Macbr.) T. Macbr.

Studied material: **MEXICO. Sonora: Loc. 1**, on *Cylindropuntia* sp., leg. AS & AG, 9-III-2009, obtained from moist chamber, 20-IV-2009 (UES 7858); **Loc. 2**, on *Opuntia* sp., leg. AS & AG, 14-III-2009, obtained from moist chamber, 15-IV-2009 (UES 7854); **Loc. 3**, on *Stenocereus thurberi* (Engelm.) Buxb., leg. AS & AG, 26-V-2009, obtained from moist chamber, 30-VI-2009 (UES 8206); **Loc. 4**, on *Lophocereus schottii* (Engelm.) Britton & Rose, leg. CS, AS & AG, 7-XI-2009, obtained from moist chamber, 4-XII-2009 (UES 8660); **Loc. 5**, on *Parkinsonia* sp., obtained from moist chamber, 20-IV-2009 (UES 7856); **Loc. 6**, on *Cylindropuntia versicolor* (Engelm.) F.M. Knuth, leg. AG & AS, 12-III-2009, obtained from moist chamber, 6-IV-2009 (UES 7845); **Loc. 7**, on *Stenocereus thurberi*, obtained from moist chamber, 14-IV-2009 (UES 7962); **Loc. 8**, on *Lophocereus schottii*, leg. AS & AG, 14-III-2009, obtained from moist chamber, 14-IV-2009 (UES 7847); **Loc. 9**, on *Cylindropuntia arbuscula* (Engelm.) F.M. Knuth, leg. AG & AS 26-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8110); **Loc. 10**, on *Parkinsonia* sp., leg. AS & AG, 12-III-2009, obtained from moist chamber, 20-IV-2009 (UES 7860).

Comments—We follow the taxonomy of Moreno & Oltra (2010), who regarded *Badhamia melanospora* Speg. as separate from *B. gracilis*. The two species differ mainly in spore morphology, with the spores >15–17 µm diam., polyhedral, and densely warty in *B. melanospora* and <12–14 µm diam, polyhedral, and with scattered warts with intervening spaces in *B. gracilis*. Both species fruit mainly on succulent plants (Castillo et al. 1996). Some studied collections were mistakenly identified as *B. melanospora* by Moreno et al. (2006) and Esqueda et al. (2013).

Badhamia spinispora (Eliasson & N. Lundq.) H.W. Keller & Schokn. FIGS 1, 5–6

Fructifications sessile, subglobose to short plasmodiocarps, white to grey, growing grouped together. Sporotheca 0.2–1 × 0.2–0.5 mm. Peridium double, calcareous, with irregular dehiscence. Hypothallus inconspicuous. Pseudocolumella irregular, calcareous. Capillitium scarce and joined by strands consisting of irregular calcareous nodules. Spores (9–)10–12 × 13–15 µm, globose to subglobose, black in mass, violaceous with a paler area under LM, ornamented with large spines. SEM shows that the spore ornamentation is formed by irregularly scattered or grouped <0.5 µm high warts.

STUDIED MATERIAL: **MEXICO. SONORA: Loc. 9,** over hare manure, leg. AG & AS, 26-V-2009; obtained from moist chamber, 30-VI-2009 (UES 8116, AH 42974).

COMMENTS—This species is characterized by its generally coprophilous habitat (although it does occasionally grow on tree bark), cespitose habit, capillitium that varies from physaroid to badhamioid, and spores that are dark in mass, spiny, and with a paler area. A study of *B. spinispora, Physarum apiculosporum* Härk., and *Badhamia verrucospora* G. Moreno & al. was published by Moreno et al. (2011).

Cited from Sonora by Esqueda et al. (2012, 2013).

Comatricha elegans (Racib.) G. Lister

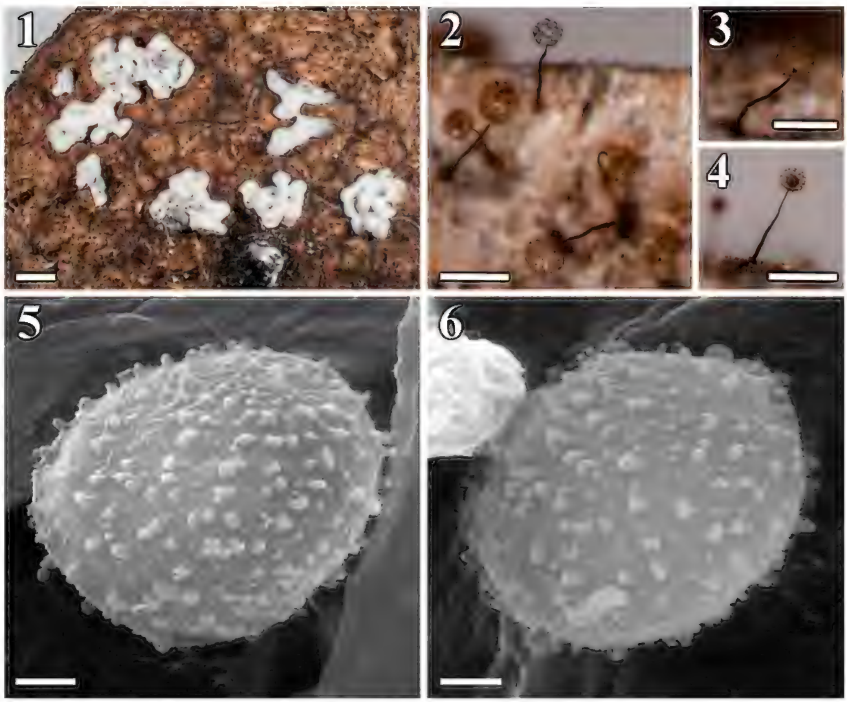
STUDIED MATERIAL: **MEXICO. SONORA: Loc. 1,** on *Olneya tesota*, leg. AG & AS, 22-V-2009, obtained from moist chamber, 3-VI-2009 (UES 8120); **Loc. 7**, leg. ML, CS, AS & AG, 17-VIII-2009, obtained from moist chamber, 7-IX-2009 (UES 8563); **Loc. 9,** 20-VIII-2009, obtained from moist chamber, 9-IX-2009 (UES 8621); **Loc. 10**, decomposing wood, 21-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8122).

COMMENTS—Lizárraga et al. (2005) published a study (including SEM photomicrographs) of *C. elegans* from Chihuahua.

Previously recorded from Sonora by Lizárraga et al. (2007, 2008a).

*****Comatricha mirabilis*** R.K. Benj. & Poitras FIGS 7–12

Fructifications stipitate, 0.7–0.9 mm tall. Sporotheca subglobose to subcylindrical, 0.4–0.6 mm diam. Peridium fleeting and persistent at the base, resembling a necklace. Stipe short, dark, 0.3–0.6 mm high. Hypothallus membranous, hyaline, as common in *Comatricha.* Columella crossing the sporotheca and forking into several branches at the apex. Capillitium parallel along the columella, dark brown with free ends, dichotomous, widened and forked at the tips that protrude at the periphery of the sporotheca. Spores dark brown in mass, 9–12 µm diam., globose to subglobose, reticulate, with walls 1–2 µm high. SEM observation reveals that the spore is reticulate and the high banded reticulum has entire and unperforated walls.



Figures 1–6. ***Badhamia spinispora*** (AH 42974): 1. Plasmodiocarps. 5–6. Spores (SEM). ***Cribraria confusa*** (AH 42976): 2–4. Sporocarps. Bars: 1 = 1 mm; 2–4 = 0.5 mm; 5–6 = 2 µm.

Studied material: **MEXICO. Sonora: Loc. 4**, on bark of *Forchhammeria watsonii*, leg. AG & AS, 27-V-2009, obtained from moist chamber, 30-VI-2009 (UES 8129, AH 42975).

Comments—*Comatricha mirabilis* is characterized by its capillitium that extends parallel along the columella without forming a network with bifurcated ends that protrude from the sporotheca and fully reticulate globose spores. It was described growing over goat manure in USA in a moist chamber (Benjamin & Poitras 1950). Later, Mandell & Blackwell (2008) gathered abundant material from tree barks of *Tamarix arabica*, *Prosopis juliflora*, *Phoenix dactylifera,* and *Cordia myxa* from a Bahrain desert in the Kingdom of Bahrain archipelago near Saudi Arabia, which they developed in a moist chamber. Andrade-Bezerra et al. (2008) described material developed on Brazilian cottontail (*Sylvilagus brasiliensis* L.) dung in a moist chamber, Poulain et al. (2011) gathered it from France on wood debris, and Schnittler et al. (2013) identified the species from arid areas in China. We obtained three sporocarps on *Forchhammeria watsonii* bark in a moist chamber. This is the first record for Mexico.

Comatricha tenerrima (M.A. Curtis) G. Lister

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota*, leg. AS & AG, 22-V-2009, obtained from moist chamber, 15-VI-2009 (UES 8124); **Loc. 9**, on decomposing wood, leg. CS, AS & AG, 6-XI-2009, obtained from moist chamber, 11-XII-2009 (UES 8685); **Loc. 10**, on decomposing wood, leg. AG & AS, 21-V-2009, obtained from moist chamber, 6-VI-2009 (UES 8130).

Comments— Lizárraga et al. (2007) published a description with SEM micrographs of *C. tenerrima* from Sonora. It has also been cited from other natural protected areas in Sonora (Lizárraga et al. 2008a,b; Esqueda et al. 2013).

****Cribraria confusa*** Nann.-Bremek. & Y. Yamam. Figs. 2–4

Fructifications small (0.3–0.9 mm high), stipitate. Sporotheca globose to subglobose, 0.1–0.2 mm diam. Stipe 0.2–0.7 mm long, dark brown, with debris. Peridium evanescent, forming a small discoid calyculus at the base beneath an open network of irregular flattened nodules covered by dictydine granules. Hypothallus membranous. Spores 7–8 µm diam., yellow in mass, light yellow under LM, globose, with a warty ornamentation.

Studied material: **MEXICO. Sonora: Loc. 3**, on palm fibers, leg. ML, CS, AS & AG, 19-VIII-2009, obtained from moist chamber, 5-IX-2009 (UES 8570); obtained from moist chamber, 7-IX-2009 (UES 8759, AH 42976 with *Arcyria cinerea* and *Paradiacheopsis* sp.); on *Guaiacum coulteri* A. Gray, obtained from moist chamber, 3-X-2009 (UES 8571).

Comments—*Cribraria confusa* has been recorded only from Chihuahua on decomposing wood of *Pinus* sp. by Lizárraga et al. (2003), who presented LM and SEM micrographs.

Cribraria violacea Rex

Studied material: **MEXICO. Sonora: Loc. 10**, on palm fibers, leg. AG, 21-V-2009, obtained from moist chamber, 15-VI-2009 (UES 8135).

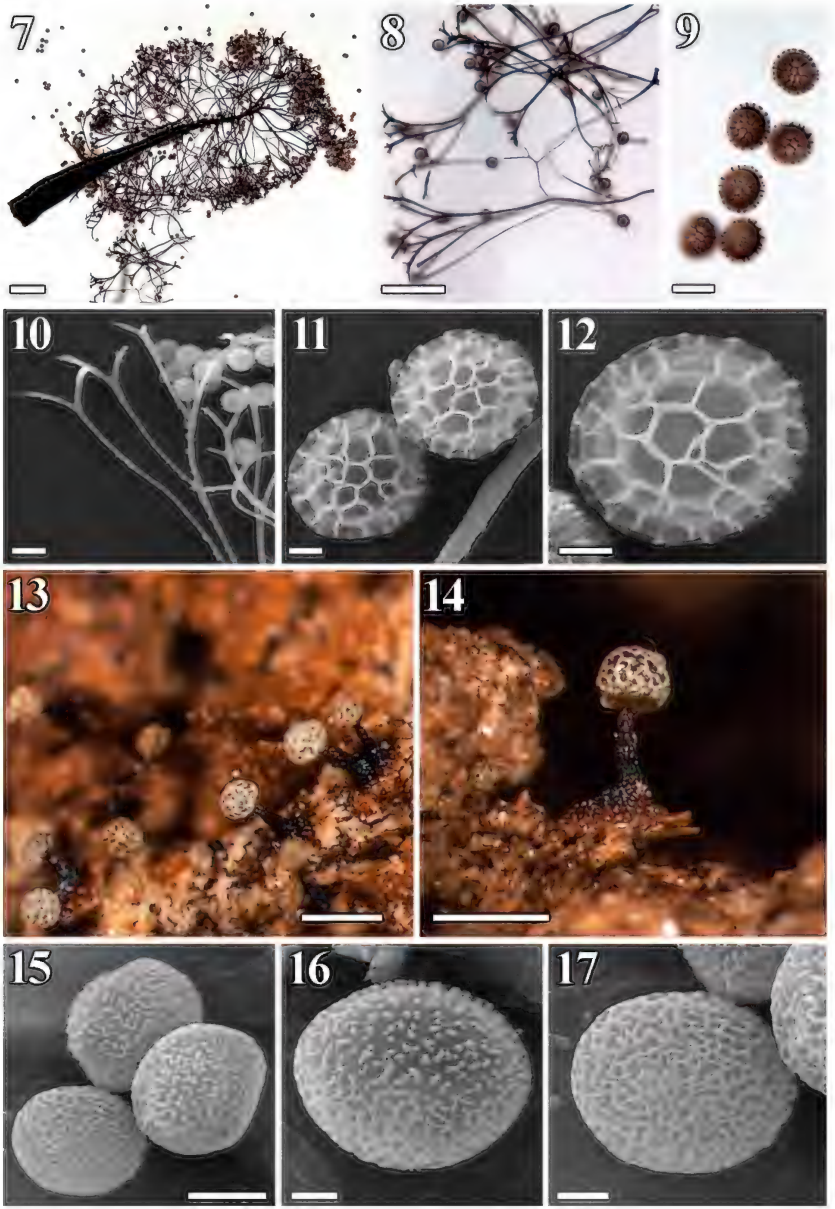
Comments— Moreno et al. (2001) published an SEM study of the spore ornamentation on material from Baja California.

Previously cited from Sonora by Moreno et al. (2006) and Lizárraga et al. (2008b).

Dictydiaethalium plumbeum (Schumach.) Rostaf.

Studied material: **MEXICO. Sonora: Loc. 1**, on decomposing wood, leg. ML, CS, AS & AG, 17-VIII-2009, obtained from moist chamber, 9-IX-2009 (UES 8572); **Loc. 5**, leg. AG & AS, 14-III-2009, obtained from moist chambers, 1-V-2009 (UES 7880).

Comments—Previously cited from Sonora by Moreno et al. (2006) and Lizárraga et al. (2008b).



Figures 7–17. ***Comatricha mirabilis*** (AH 42975): 7. Sporocarp. 8. Capillitium. 9. Spores. 10. Capillitium endings (detail, SEM). 11–12. Spores by SEM. ***Didymium eremophilum*** (AH 42977): 13. Sporocarps. 14. Sporocarp (detail). 15–17. Variability of spore ornamentation (SEM). Bars: 7 = 0.1 mm; 8 = 50 µm; 9 = 10 µm; 13–14 = 0.5 mm; 15 = 5 µm; 16–17 = 2 µm.

Didymium dubium Rostaf.

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota*, leg. AS & AG, 9-III-2009, obtained from moist chamber, 20-IV-2009 (UES 7888); **Loc. 2**, on *Prosopis glandulosa*, leg. ML, CS, AS & AG, 19-VIII-2009, obtained from moist chamber, 9-IX-2009 (UES 8755); **Loc. 5**, on *Prosopis glandulosa,* leg. AS & AG, 14-III-2009, obtained from moist chamber, 9-IV-2009 (UES 7886); **Loc. 6**, on *Prosopis glandulosa,* leg. AG & AS 12-III-2009, obtained from moist chamber, 25-IV-2009 (UES 7889); **Loc. 8**, on *Cylindropuntia* sp., leg. AG & AS, 14-III-2009, obtained from moist chamber, 14-IV-2009 (UES 7882); **Loc. 9**, on *Cylindropuntia arbuscula,* leg. CS, AS & AG, 6-XI-2009, obtained from moist chamber, 26-XI-2009 (UES 8692).

Comments—Moreno et al. (2001) published a SEM study of the spore ornamentation. Previously cited from Sonora by Moreno et al. (2006).

****Didymium eremophilum*** M. Blackw. & Gilb. Figs 13–17

Fructifications stipitate, gregarious, 0.2–0.9 mm tall. Sporotheca globose to subglobose, 0.1–0.3 mm diam., whitish with lighter zones, with a dark disc at the base. Stipe robust, 0.1–0.5 mm tall, reddish-brown to darkish brown, wider and darker at the base. Hypothallus membranous, discoid, concolorous with stipe. Peridium double, with the outer membrane formed by calcareous crosshairs, the inner membranous with iridescent blue tones. Capillitium and columella absent. Spores globose to subglobose, black in mass, violaceous by LM, 9–10 µm diam., with a reticulate hemisphere and the opposite warty (as confirmed by SEM; see Moreno & Mitchell 2013).

Studied material: **MEXICO. Sonora: Loc. 4**, leg. CS, AS & AG, 7-IX-2009, obtained from moist chamber, 4-XII-2009 (UES 8698); **Loc. 8**, on *Lophocereus schottii*, leg. AG & AS, 21-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8141, AH 42977); obtained from moist chamber, 15-VI-2009 (UES 8142); obtained from moist chamber, 19-VI-2009 (UES 8143); obtained from moist chamber, 22-VI-2009 (UES 8144); obtained from moist chamber, 30-VI-2009 (UES 8145); leg. CS, AS & AG, 6-XI-2009, obtained from moist chamber, 30-XI-2009 (UES 8699); obtained from moist chamber, 11-XII-2009 (UES 8700); obtained from moist chamber, 12-XII-2009 (UES 8701); obtained from moist chamber, 12-XII-2009 (UES 8702); **Loc. 9**, on cactus remains, leg. AG & AS, 26-V-2009, obtained from moist chamber, 15-VI-2009 (UES 8146).

Comments—*Didymium eremophilum* is a succulenticolous species adapted to arid environments (Blackwell & Gilbertson 1980b), characterized by its small size, stipitate sporocarps, crystalline lime, lack of capillitium, partly reticulate verrucose spores, and succulenticolous habitat (Moreno & Mitchell 2013).

Didymium eremophilum specimens, very difficult to find due to their small size, have thus far been found only from moist chamber. Previously recorded in Mexico only from Puebla (Estrada-Torres et al. 2009), our study provides a first record for the Sonoran mycobiota.

Didymium squamulosum (Alb. & Schwein.) Fr.

Studied material: **MEXICO. Sonora: Loc. 4**, on deer manure, leg. AG & AS 12-III-2009, obtained from moist chamber, 1-V-2009 (UES 7881); **Loc. 7**, on *Stenocereus thurberi,* leg. AG & AS, 11-III-2009, obtained from moist chamber, 6-IV-2009 (UES 7890).

Comments—Lizárraga et al. (1999a) published a SEM study of the spore ornamentation. Widely cited for Mexico (Moreno et al. 2007), *D. squamulosum* has been previously cited for Sonora by Moreno et al. (2006) and Lizárraga et al. (2008b).

Didymium sturgisii Hagelst. Figs 18–23

Studied material: **MEXICO. Sonora: Loc. 7**, on *Prosopis glandulosa,* leg. AG & AS, 11-III-2009, obtained from moist chamber, 8-IV-2009 (UES 7884, AH 42978).

Comments—This species is characterized by its dirty white to whitish grey flattened to subglobose fructifications (occasionally with small superficial orifices) and sporocarp interiors with erect pillars or internally traversing trabeculae. Moreno et al. (2001) published an SEM study of the capillitium, pillars, and spores.

Didymium sturgisii has been recorded from Baja California (Moreno et al. 2007), Chihuahua (Salazar-Márquez et al. 2014), Puebla (Estrada-Torres et al. 2009), and Sonora (Esqueda et al. 2012, 2013).

Echinostelium apitectum K.D. Whitney

Studied material: **MEXICO. Sonora: Loc. 1**, on *Prosopis glandulosa* leg. AS & AG, 9-III-2009, observed in moist chamber, 28-III-2009, 4-IV-2009 (UES 7892); **Loc. 3**, on *Stenocereus thurberi*, 19-VIII-2009, obtained in moist chamber, 2-IX-2009, 5-IX-2009 (UES 8589); **Loc. 4**, on *Olneya tesota,* leg. AG & AS, 3-VI-2009, obtained from moist chamber, 17-VI-2009 (UES 8149); **Loc. 5**, on decomposing wood, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 2-IX-2009, 5-IX-2009 (UES 8581); **Loc. 6**, on *Prosopis glandulosa*, leg. AG & AS, 12-III-2009, observed in moist chamber, 28-III-2009, 2-IV-2009 (UES 7902); **Loc. 7**, on *Prosopis glandulosa*, leg. AG & AS, 22-V-2009, observed in moist chamber, 3-VI-2009, 22-VI-2009 (UES 8151); **Loc. 9,** *Carnegiea gigantea*, leg. ML, CS, AS & AG, 20-VIII-2009, observed in moist chamber, 2-IX-2009, 7-IX-2009 (UES 8584); **Loc. 10**, on *Prosopis glandulosa*, leg. CS, AS & AG, 3-XI-2009, observed in moist chamber, 9-XI-2009, 30-XI-2009 (UES 8703).

Comments—Previously cited from Sonora by Lizárraga et al. (2007, 2008a).

Echinostelium arboreum H.W. Keller & T.E. Brooks

Studied material: **MEXICO. Sonora: Loc. 1**, on decomposing wood, leg. AG & AS, 22-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8106 with *Arcyria cinerea*).

Comments—Moreno et al. (2001) published a SEM study of the sporocarp and spores. Previously cited from Sonora by Esqueda et al. (2013), *E. arboreum* has been widely reported from Mexico (Moreno et al. 2007).

Echinostelium colliculosum K.D. Whitney & H.W. Keller

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota,* leg. AS & AG, 9-III-2009, 28-III-2009, obtained from moist chamber, 4-IV-2009 (UES 7891); **Loc. 5**, on *Guaiacum coulteri*, 14-III-2009, obtained from moist chamber, 4-IV-2009 (UES 7893); on *Olneya tesota,* observed in moist chamber, 4-IV-2009 (UES 7894); on *Prosopis glandulosa,* obtained from moist chamber, 8-IV-2009 (UES 7895); on *Parkinsonia* sp., leg. AG & AS, 26-V-2009, obtained from moist chamber, 11-VI-2009 (UES 8152); **Loc. 6**, on *Cylindropuntia arbuscula*, leg. AS & AG, 27-V-2009, 3-VI-2009, obtained from moist chamber, 11-VI-2009 (UES 8153); on *Parkinsonia* sp., leg. ML, CS, AS & AG, 19-VIII-2009, in moist chamber, 2-IX-2009, 5-IX-2009 (UES 8588); **Loc. 7**, on *Parkinsonia* sp., leg. AS & AG, 11-III-2009, in moist chamber, 9-IV-2009 (UES 7901); on *Encelia farinosa* A. Gray ex Torr., 22-V-2009, 3-VI-2009, in moist chamber, 11-VI-2009 (UES 8150); on decomposing wood, leg. ML, CS, AS & AG, 17-VIII-2009, in moist chamber, 2-IX-2009, 5-IX-2009 (UES 8591); **Loc. 9**, on *Guaiacum coulteri*, 11-III-2009, 28-III-2009, in moist chamber, 4-IV-2009 (UES 7896); on *Olneya tesota,* in moist chamber, 4-IV-2009 (UES 7898); on *Cylindropuntia arbuscula*, in moist chamber, 4-IV-2009 (UES 7899); leg. ML, CS, AS & AG, 20-VIII-2009, in moist chamber, 2-IX-2009, 7-IX-2009 (UES 8583); **Loc. 10**, on *Cylindropuntia* sp., leg. AS & AG, 12-III-2009, 4-IV-2009 (UES 7900); leg. ML, CS, AS & AG, 18-VIII-2009, in moist chamber, 9-IX-2009 (UES 8582); **Loc. 10**, on *Cylindropuntia* sp., leg. AS & AG, 12-III-2009, 4-IV-2009 (UES 7900).

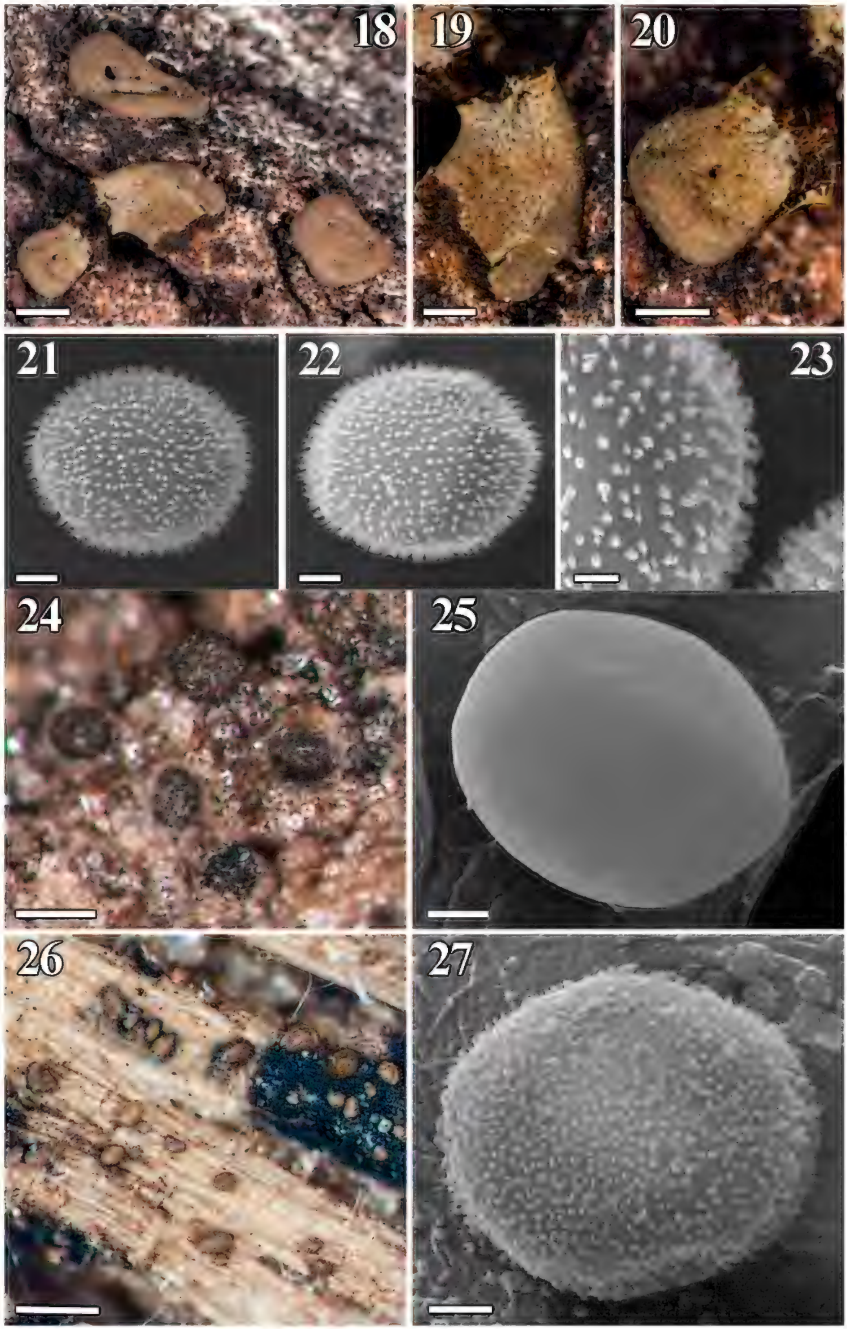
Comments—This species is characterized by its (60–)75–90 µm high stipitate sporocarps, globose 9–13 µm diam. pale yellow spores that have a spine-like ornamentation and articular spore wall. A LM study of sporocarps and spores by Lizárraga et al. (2003) and SEM study by Moreno et al. (2001) have been published.

Echinostelium colliculosum has been recorded for Baja California Sur and Chihuahua (Moreno et al. 2007), Oaxaca and Puebla (Estrada-Torres et al. 2009), and Sonora (Esqueda et al. 2013).

Echinostelium minutum de Bary

Studied material: **MEXICO. Sonora: Loc. 1**, on *Prosopis glandulosa*, leg. ML, CS, AS & AG, 17-VIII-2009, in moist chamber, 2-IX-2009, 7-IX-2009 (UES 8580); **Loc. 3**, on *Stenocereus thurberi*, leg. ML, CS, AS & AG, 17-VIII-2009, 7-IX-2009 (UES 8590); **Loc. 7**, on *Prosopis glandulosa*, leg. AG, 22-05-2009, obtained from moist chamber, 11-06-2009

Figures 18–27. ***Didymium sturgisii*** (AH 42978): 18. Fructifications flattened with some calcium carbonate granules. 19. Sporocarp (detail of small superficial orifices). 20. Capillitium and erect pillars. 21–22. Spores (SEM). 23. Spore ornamentation (detail, SEM). ***Licea denudescens*** (AH 42979): 24. Sporocarps. 25. Spore (SEM). ***Licea succulenticola*** (AH 42980): 26. Sporocarps. 27. Spore (SEM). Bars: 18–19 = 0.5 mm; 20 = 0.25 mm; 21–22 = 2 µm; 23 = 1 µm.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(UES 8154); **Loc. 9**, **on** *Carnegiea gigantea*, 9-IX-2009 (UES 8585); **Loc. 10**, on *Prosopis glandulosa,* leg. CS, AS & AG, 3-XI-2009, in moist chamber, 9-XI-2009, II-2009 (UES 8704).

Comments— Moreno et al. (2001) published a SEM study of the sporocarp and spores. Previously reported from Sonora by Moreno et al. (2006).

Enerthenema papillatum (Pers.) Rostaf.

Studied material: **MEXICO. Sonora: Loc. 4**, on *Forchhammeria watsonii*, leg. ML, CS, AS & AG, 19-VIII-2009, obtained in moist chamber, 5-IX-2009 (UES 8758).

Comments—Lizárraga et al. (1999a) published a SEM study of the spores from collections from Baja California, Mexico. Widely recorded in Mexico (Moreno et al. 2007), *E. papillatus* was recorded previously from Sonora by Moreno et al. (2006).

Fuligo cinerea (Schwein.) Morgan

Studied material: **MEXICO. Sonora: Loc.10**, on sheep manure, leg., AG, 21-V-2009, obtained from moist chamber, 30-VI-20009 (UES 8118).

Comments—Sometimes similar in size as some *Badhamia* species (e.g., *B. spinispors, B. verrucospora* G. Moreno et al.), *Fuligo cinerea* is separated by its ovoid spores covered by small warts that join together to form a reticulum. Cited previously for Sonora by Braun & Keller (1986) and Lizárraga et al. (2007).

Fuligo septica (L.) F.H. Wigg.

Studied material: **MEXICO. Sonora: Loc. 9**, on soil, leg. ML, 11-III-2009 (UES 8677).

Comments—Lizárraga et al. (1999a) published a SEM study of the spores. *Fuligo septica* has a wide distribution in Mexico (Moreno et al. 2007) and has been cited for Sonora by Pérez-Silva et al. (2001) and Lizárraga et al. (2007, 2008a,b).

Licea biforis Morgan

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota,* leg. AG & AS, 9-III-2009, obtained in moist chamber, 6-IV-2009 (UES 7905); **Loc. 2**, on *Prosopis glandulosa*, leg. AS & AG, 14-III-2009, obtained in moist chamber, 13-IV-2009 (UES 7908); **Loc. 3**, on *Acacia* sp., 26-V-2009, obtained in moist chamber, 15-VI-2009 (UES 8159); **Loc. 5**, on *Guaiacum coulteri* bark, leg. AS & AG 14-III-2009, obtained in moist chamber, 20-IV-2009 (UES 7913); **Loc. 6**, on *Prosopis glandulosa*, leg. AG & AS, 27-V-2009, obtained in moist chamber, 15-VI-2009 (UES 8162); **Loc. 9**, on *Olneya tesota,* leg. AG & AS, 11-III-2009, obtained in moist chamber, 1-V-2009 (UES 7915); **Loc. 10**, leg. CS, AS & AG, 3-XI-2009, obtained in moist chamber, 12-XII-2009 (UES 8716).

Comments—Lizárraga et al. (1998, 1999b) published SEM studies of the spores. Widely distributed in Mexico (Moreno et al. 2007), *Licea biforis* was previously cited from Sonora by Moreno et al. (2006).

Licea denudescens H.W. Keller & T.E. Brooks Figs 24–25

Fructifications sessile, globose to subglobose, 0.1–0.3 mm diam., 0.1 mm height, brown to blackish. Peridium membranous with some iridescent tones, internally ornamented with small buds. Spores violaceous in mass, yellowish brown by LM, 10–11 µm diam., globose to subglobose, walls thick with pale (thinner) areas, smooth by SEM.

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota*, leg. AS & AG, 9-III-2009, obtained in moist chamber, 8-IV-2009 (UES 7916, AH 42979); **Loc. 5**, on *Olneya tesota*, leg. CS, AS & AG, 6-XI-2009, obtained in moist chamber, 12-XII-2009 (UES 8711); **Loc. 7**, on *Parkinsonia* sp., 11-III-2009, obtained in moist chamber, 20-IV-2009 (UES 7918); obtained in moist chamber, 1-V-2009 (UES 7917); **Loc. 8**, on *Acacia greggii* A. Gray, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 3-X-2009 (UES 8605); **Loc. 9**, on cactus remains, 11-III-2009, observed in moist chamber, 15-IV-2009 (UES 7919).

Comments—*Licea denudescens* is diagnosed by its thick-walled spores with thinner areas and papillae found on the inside of the peridium. It has been cited in Mexico from Puebla (Estrada-Torres et al. 2009), Chihuahua (Salazar-Márquez et al. 2014), and Sonora (Esqueda et al. 2013).

Licea kleistobolus G.W. Martin

Studied material: **MEXICO. Sonora: Loc. 8**, on *Acacia greggii* bark, leg. ML, CS, AS & AG, 18-VIII-2009, observed in moist chamber, 7-IX-2009 (UES 8608).

Comments—Moreno et al. (2001) published a SEM study of *Licea kleistobolus*, which is characterized by its circular to subglobose fructifications with a characteristic operculum and warted spores with groups of denser warts. Cited previously from Sonora by Moreno et al. (2006) and Lizárraga et al. (2007).

Licea succulenticola Mosquera, Lado, Estrada & Beltrán-Tej. Figs 26–27

Fructifications sessile, yellowish to dark-yellow at mature, dispersed to gregarious, subglobose, oval to elongated, 0.1–0.3 mm long, 0.1–0.2 mm diam., and 0.1 mm tall, with an apical and longitudinal dehiscence. Peridium membranous, ornamented on its inner part with small warts. Spores yellow in mass, light yellow by LM, globose to subglobose, (11–)12–14 µm diam., with a lightly paler area, with a slightly warty ornamentation. SEM examination shows that the spore ornamentation is formed by small croziers more or less uniformly distributed over the in spore surface.

Studied material: **MEXICO. Sonora: Loc. 2**, on *Opuntia* sp., 5-XI-2009, observed in moist chamber, 14-XII-2009 (UES 8712); **Loc. 3**, on *Cylindropuntia* sp., leg. AS & AG,

14-III-2009, obtained in moist chamber, 28-III-2009, 20-IV-2009 (UES 7924); obtained in moist chamber, 25-IV-2009 (UES 7925, 7926); **Loc. 4**, on *Lophocereus schottii*, leg. AS & AG, 12-III-2009, obtained from moist chamber, 20-IV-2009 (UES 7923); **Loc. 6**, on *Lophocereus schottii*, leg. CS, AS & AG, 7-XI-2009, observed in moist chamber, 9-XI-2009, 9-XII-2009 (UES 8726); **Loc. 7**, on *Lophocereus schottii*, leg. AS & AG, 11-III-2009, observed in moist chamber, 28-III-2009, 20-IV-2009 (UES 7927); obtained in moist chamber, 1-V-2009 (UES 7928, 7929); on *Stenocereus thurberi*, leg. ML, CS, AS & AG, 17-VIII-2009, observed in moist chamber, 2-IX-2009, 7-IX-2009 (UES 8747, AH 42980); obtained in moist chamber, 14-IX-2009 (UES 8602); obtained in moist chamber, 21-IX-2009 (UES 8604); obtained in moist chamber, 21-IX-2009 (UES 8603); **Loc. 8**, on *Lophocereus schottii*, leg. ML, CS, AS & AG, 18-VIII-2009, observed in moist chamber, 2-IX-2009, 9-IX-2009 (UES 8594); obtained in moist chamber, 14-IX-2009 (UES 8595); obtained in moist chamber, 24-IX-2009 (UES 8596); obtained in moist chamber, 30-IX-2009 (UES 8597); obtained in moist chamber, 5-X-2009 (UES 8598, AH 42981); on *Stenocereus thurberi*, leg. CS, AS & AG, 6-XI-2009, observed in moist chamber, 9 -XI-2009, 14-XII-2009 (UES 8725); **Loc. 10**, on *Cylindropuntia arbuscula*, leg. AG & AS, 12-III-2009, obtained in moist chamber, 20-IV-2009 (UES 7920); on cactus remains, obtained in moist chamber, 20-IV-2009 (UES 7921); obtained in moist chamber, 1-V-2009 (UES 7922); on *Lophocereus schottii*, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 2-IX-2009, 17-IX-2009 (UES 8599); obtained in moist chamber, 30-IX-2009 (UES 8600); obtained in moist chamber, 30-IX-2009 (UES 8601).

Comments—*Licea succulenticola* resembles *L. pumila* G.W. Martin & R.M. Allen (Martin & Alexopoulos 1969) in sporothecal form and spore colour. (see Mosquera et al. 2003 for differences). Previously recorded from Hidalgo, Morelos, Puebla, and Tlaxcala (Mosquera et al. 2003), Puebla (Estrada-Torres et al. 2009), Sonora (Esqueda et al. 2013), and Chihuahua (Salazar-Márquez et al. 2014).

Paradiacheopsis fimbriata (G. Lister & Cran) Hertel ex Nann.-Bremek. Figs 28–30

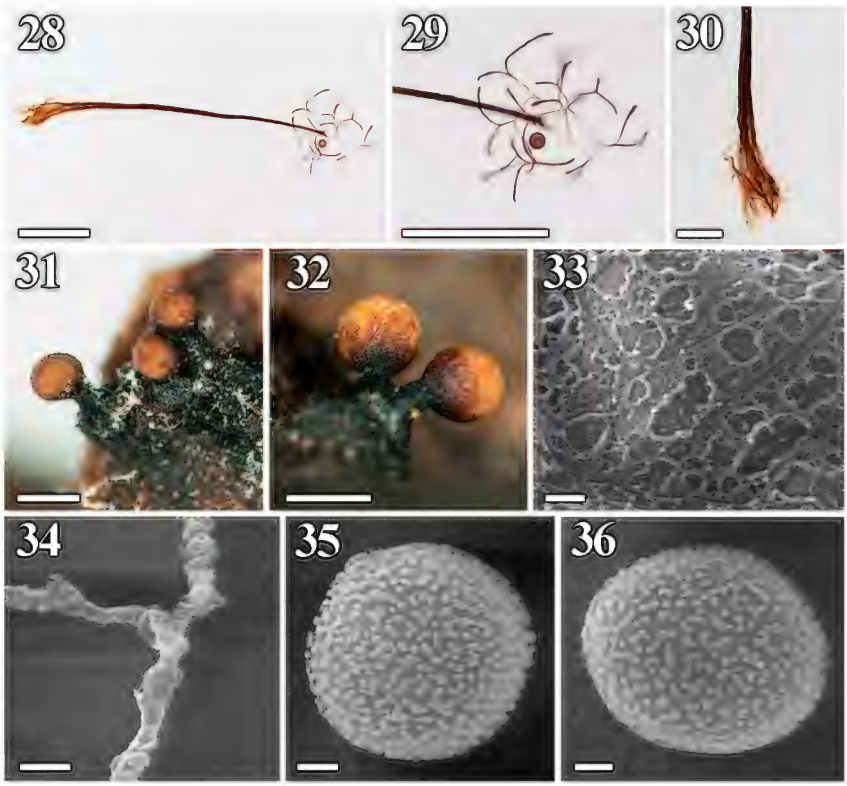
Studied material: **MEXICO. Sonora: Loc. 3**, on *Cylindropuntia* sp., leg. AG, 14-III-2009, observed in moist chamber, 28-III-2009, 13-IV-2009 (UES 7943, AH 42982).

Comments—Widely distributed in Mexico (Moreno et al. 2007), *P. fimbriata* was reported from Sonora by Moreno et al. (2006).

Perichaena depressa Lib.

Studied material: **MEXICO. Sonora: Loc. 1**, on rabbit manure, leg. ML, CS, AS & AG, 17-VIII-2009, obtained in moist chamber, 9-X-2009 (UES 8628); **Loc. 9**, on horse manure, 20-VIII-2009, obtained in moist chamber, 5-X-2009 (UES 8629).

Comments—Lizárraga et al. (1998) and Moreno et al. (2001) published SEM studies of spores and capillitium, comparing type material with samples from Baja California and Sinaloa. Widely distributed throughout Mexico (Moreno et al. 2007), *P. depressa* was previously cited for Sonora by Moreno et al. (2006).



FIGURES 28–36. ***Paradiacheopsis fimbriata*** (AH 42982): 28. Sporocarp. 29. Sporotheca (detail). 30. Fiber stalk (detail). ***Perichaena stipitata*** (AH 42983): 31–32. Stipitate sporocarp (detail). 33. Peridial inner surface (detail). 34. Capillitium with irregular outline. 35–36. Spores (SEM). Bars: 28–29 = 0.1 mm; 30 = 0.02 mm; 31–32 = 0.5 mm; 33, 35–36 = 1 µm; 34 = 5 µm.

**Perichaena luteola* (Kowalski) Gilert

STUDIED MATERIAL: **MEXICO. SONORA: Loc. 10**, on cow manure, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 27-IX-2009 (UES 8749); 3-X-2009 (UES 8627).

COMMENTS—The above collections have 10–13 µm diam. globose to subglobose spores that are yellow in mass (lighter yellow by LM) and with a verruculose episporium and a yellow 2–3µm diam. capillitium with smooth surface. Lizárraga et al. (1999b) published a SEM study of material from Baja California. *Perichaena luteola* is widely distributed in Mexico (Moreno et al. 2007).

Perichaena stipitata Lado, Estrada & D. Wrigley FIGS 31–36

Fructifications short-stipitate, solitary to gregarious, 0.3–0.8 mm tall. Stipe robust, cylindrical, black, 0.1–0.2 mm long. Sporotheca globose to subglobose, rarely obpyriform, yellow, 0.2–0.5 mm diam. Peridium brownish on the base, inner surface ornamented with small rings by LM and SEM. Hypothallus dark, membranous and irregular. Capillitium sparse, formed by yellowish branched filaments of approximately 2 µm diam., flexible, of irregular outline. Spores yellow in mass, light yellow by LM, 12–15 µm diam., globose, warted. SEM examination shows that the spore is ornamented by warts with smooth apices and which sometimes join into small groups.

STUDIED MATERIAL: **MEXICO. SONORA: Loc. 9**, on cactus remains, leg. AG & AS, 26-V-2209, obtained in moist chamber, 30-VI-2009 (UES 8210, AH 42983); on *Lophocereus schottii*, leg. ML, CS, AS & AG, 20-VIII-2009, obtained in moist chamber, 17-IX-2009 (UES 8762); obtained in moist chamber, 24-IX-2009 (UES 8761); obtained in moist chamber, 3-X-2009 (UES 8760, AH 42984); obtained in moist chamber, 9-X-2009 (UES 8643); **Loc. 10**, on *Lophocereus schottii*, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 5-X-2009 (UES 8642).

COMMENTS—Stipitate fructifications and an inner peridium ornamented by small rings characterize this succulenticolous species. First described as new to science by Estrada-Torres et al. (2009) from Puebla and Queretaro, Mexico, *P. stipitata* was later described from Arizona (USA) by Moreno & Mitchell (2013), who published a colour plate showing its development and morphological characteristics. A new record for Sonora.

Perichaena vermicularis (Schwein.) Rostaf.

STUDIED MATERIAL: **MEXICO. SONORA: Loc. 1**, on *Carnegiea gigantea*, leg. CS, AS & AG, 5-XI-2009, obtained in moist chamber, 11-XII-2009 (UES 8734); **Loc. 2**, on *Cylindropuntia arbuscula*, leg. AS & AG, 22-V-2009, obtained in moist chamber, 25-VI-2009 (UES 8191); **Loc. 3**, on *Cylindropuntia* sp., leg. AS & AG 14-III-2009, obtained in moist chamber, 15-IV-2009 (UES 7953); **Loc. 4**, on *Forchhammeria watsonii*, leg. AG & AS, 12-III-2009, obtained in moist chamber, 3-IV-2009 (UES 7944); **Loc. 6**, *Parkinsonia* sp., 19-VIII-2009, obtained in moist chamber, 24-IX-2009 (UES 8633); **Loc. 7**, on *Cactaceae* remains, 11-III-2009, obtained in moist chamber, 8-IV-2009 (UES 7947); obtained in moist chamber, 20-IV-2009 (UES 7948); **Loc. 8**, on *Cactaceae* remains, obtained in moist chamber, 18-VIII-2009, 17-IX-2009 (UES 8631).

COMMENTS—Moreno et al. (2001) published a SEM study of spores and capillitium in collections from Baja California that grew cespitose in moist chamber over different substrates. Widely distributed in Mexico (Moreno et al. 2007), *P. vermicularis* was previously reported from Sonora by Moreno et al. (2006).

Physarum album (Bull.) Chevall.

Studied material: **MEXICO. Sonora: Loc. 7**, on *Prosopis glandulosa*, leg. AG & AS, 17-VIII-2009, obtained in moist chamber, 9-IX-2009 (UES 8651); **Loc. 10**, on *Prosopis glandulosa*, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 7-IX-2009 (UES 8650).

Comments—Lizárraga et al. (1999b) published a SEM study of spore ornamentation in collections from Baja California. Widely distributed in Mexico (Moreno et al. 2007) and previously reported for Sonora (Lizárraga et al. 2007, 2008b), *Physarum album* frequently grows on many different substrates when cultivated in moist chamber.

Physarum decipiens M.A. Curtis

Fructifications sessile, dispersed to grouped, subglobose or forming short plasmodiocarp. Sporotheca yellow-greenish, 0.3–1.2 × 0.2–0.6 mm, membranous, peridium encrusted with calcium carbonate. Capillitium physaroid. Spores 11–13 µm diam., subglobose to globose, violaceous and slightly warty.

Studied material: **MEXICO. Sonora: Loc. 1**, on *Prosopis glandulosa*, leg. AG & AS, 9-III-2009, obtained in moist chamber, 6-IV-2009 (UES 7967); 22-V-2009, obtained in moist chamber, 11-VI-2009 (UES 8193); **Loc. 2**, on *Prosopis glandulosa*, leg. AS & AG 14-III-2009, obtained in moist chamber, 8-IV-2009 (UES 7970); **Loc. 4**, on *Forchhammeria watsonii*, leg. ML, CS, AS & AG, 19-VIII-2009, obtained in moist chamber, 7-IX-2009 (UES 8649).

Comments—Morphologically, *P. decipiens* is highly variable. Previously cited from Chihuahua (Lizárraga et al. 2003), Oaxaca and Puebla (Estrada-Torres et al. 2009), Tlaxcala (Rodríguez-Palma et al. 2002), and recently Sonora (Esqueda et al. 2012, 2013).

Physarum leucophaeum Fr.

Studied material: **MEXICO. Sonora: Loc. 1**, on *Olneya tesota*, leg. AS & AG, 22-V-2009, obtained in moist chamber, 25-VI-2009 (UES 8194); **Loc. 5**, on *Parkinsonia* sp., leg. AS & AG 14-III-2009, obtained in moist chamber, 1-V-2009 (UES 7966); **Loc. 6**, on *Encelia farinosa*, 27-V-2009, obtained in moist chamber, 22-VI-2009 (UES 8198); **Loc. 8**, on *Larrea tridentata* Coville, leg. AS & AG, 21-V-2009, obtained in moist chamber, 30-VI-2009 (UES 8202); **Loc. 10**, on *Guaiacum coulteri*, leg. ML, CS, AS & AG, 18-VIII-2009, obtained in moist chamber, 9-X-2009 (UES 8653).

Comments— Moreno et al. (2001) published a SEM study of spore ornamentation in collections from Baja California. The recently named species from arid regions of Eurasia and North and South America, *Physarum pseudonotabile* Novozh. et al. (Novozhilov et al. 2013), is very close to *P. notabile*

T. Macbr. and *P. leucophaeum;* distinguishing the three species is complex and additional ecological, morphological, and molecular studies are needed. *Physarum leucophaeum* is widely distributed in Mexico (Moreno et al. 2007), common when cultured in moist chamber. Previously cited from Sonora by Lizárraga et al. (2007, 2008b).

Acknowledgments

This research was made possible by support from the following projects: CONABIO (Proyecto GT016). We wish to express our gratitude to Dr. S.L. Stephenson (University of Arkansas, U.S.A.) and Mr. D.W. Mitchell (Upper Hartfield, U.K.) for reviewing the manuscript and providing useful comments. We also extend our thanks to Mr. A. Priego and Mr. J.A. Pérez (Electron Microscopy Service, University of Alcalá de Henares) for their invaluable help with the SEM. We also thank Luis Monje (Department of Drawing and Scientific Photography, University of Alcalá de Henares) for his help in digitally preparing the photographs, and we are grateful to Dr. J. Rejos, curator of the AH herbarium for his assistance with the specimens examined in the present study. M. Lizárraga extends his gratitude to Dr. Alejandro Martínez Martínez (Jefe del Dpto. de Ciencias Químico Biológicas, ICB-UACJ) for his assistance in obtaining financial support. C. Salazar thanks CONACYT for her scholarship to obtain a master's degree.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.165

Volume 130, pp. 165–171 *January–March 2015*

New taxa of *Ambomucor* (*Mucorales, Mucoromycotina*) from China

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Abstract — A new species and a new variety, *A. ovalisporus* and *A. seriatoinflatus* var. *longior,* are described from soils in northwest China. A key to all known taxa of *Ambomucor* accompanies descriptions and line drawings of the two new taxa. The maximum growth temperature used as an auxiliary criterion in classification of *Mucorales* is also discussed.

Key words — morphology, taxonomy, *Mucoraceae*, *Zygomycota*

Introduction

The genus *Ambomucor* (*Mucorales*), typified by *A. seriatoinflatus* R.Y. Zheng & X.Y. Liu, was introduced recently (Zheng & Liu 2014), together with *A. seriatoinflatus* var. *brevior* R.Y. Zheng & X.Y. Liu and *A. clavatus* R.Y. Zheng & X.Y. Liu. These taxa were found in northwest China. Recently, two new *Ambomucor* taxa were found in the northwest regions (Tibet and Shaanxi Province) of the country and are described here as *A. ovalisporus* and *A. seriatoinflatus* var. *longior*. All *Ambomucor* species and varieties are characterized by simultaneously having two kinds of sporangia: 1) fertile sporangia containing a columella and sporangiospores and 2) aborted sporangia that lack spores and a columella and which proliferate in chains. The criteria for differentiating the taxa of the enlarged *Ambomucor* are almost the same as previously reported (Zheng & Liu 2014). A key to all *Ambomucor* taxa is presented.

Materials & methods

Isolation

A small piece of moss, humus, or dung was soaked and stirred with sterilized distilled water. Different dilutions were poured with PDA media to make plates containing the

antibiotic streptomycin. The isolation plates were cultivated in the basement (5–10°C, dark) and at room temperature (about 20°C, natural solar day/night cycle). When colonies were visible under stereomicroscopes, single colonies were transferred to PDA slant tubes for subsequent purification and identification.

Cultures

Vouchers of the two strains studied are preserved in culture collections and the herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China: living cultures in the Culture Collection of the State Key Laboratory of Mycology (Am-) and the China General Microbiological Culture Collection Center (CGMCC); and dried cultures in the Herbarium Mycologicum Academiae Sinicae (HMAS).

Media & cultivation

PDA (pH 7) and modified SMA (dextrose 20 g, asparagine 2 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, thiamine chloride 0.5 mg, agar 20 g in 1000 mL distilled water, pH 7; Hesseltine & Ellis 1973) were used for morphological studies. PDA was also used for the mating experiments (with 3% lecithin added) and the detection of maximum growth temperatures. Cultivation durations and temperatures were: 1) 5–7(–10) days at 18°C for describing morphological features; 2) 4–7 days at 25–35°C for determining maximum growth temperatures; and 3) 7–20 days at 18°C for observing sexual reactions. Seven-day cultures in test tubes were used to measure the height of colonies.

Taxonomy

Ambomucor ovalisporus X.Y. Liu & R.Y. Zheng, **sp. nov.** Fig. 1

MycoBank MB518427

Differs from all other *Ambomucor* spp. by its higher colonies, its wider hyphae, its fewer sporangiophore types, its pyriform to obovoid apophysate fertile sporangia, and its globose to oval non-vacuolate sporangiospores.

Type: China, Tibet, Nyingchi, Bomê, on wild animal dung, 17 July 2004, Xiao-yong Liu (**Holotype**, HMAS 98956-1; **isotypes**, HMAS 98956-2,-3,-4,-5,-6,-7,-8; ex-type cultures, Am-14, CGMCC 3.06806).

Etymology: *ovalisporus*, referring to the oval shape of the sporangiospores.

Colonies on SMA and PDA at first granulate and then floccose, 9 cm diameter in 7 days at 18°C, up to 2.5 cm high, at first dirty white and then 'Pinkish Buff' (Ridgway XXIX), reverse at first dirty white and then 'Pinkish Cinnamon' (Ridgway XXIX). Hyphae 21.5–66.5(–80.5) µm diameter, remaining equal or narrowed at septa, hyaline to yellow-brown. Stolons absent. Rhizoids scanty, arising from various parts of the sporangiophores and from fertile and aborted sporangia. Sporangiophores erect or recumbent, arising directly from substrate or hyphae, forming both fertile and aborted sporangia always in a sympodial pattern, (37–)322–1416(–3476) µm long, 5.5–79 µm diameter, usually equal or subequal in width throughout, always constricted just beneath the terminal fertile sporangia, hyaline to brown, with or without granular contents, always verrucate; main axes mostly simple, single, rarely with 2–3

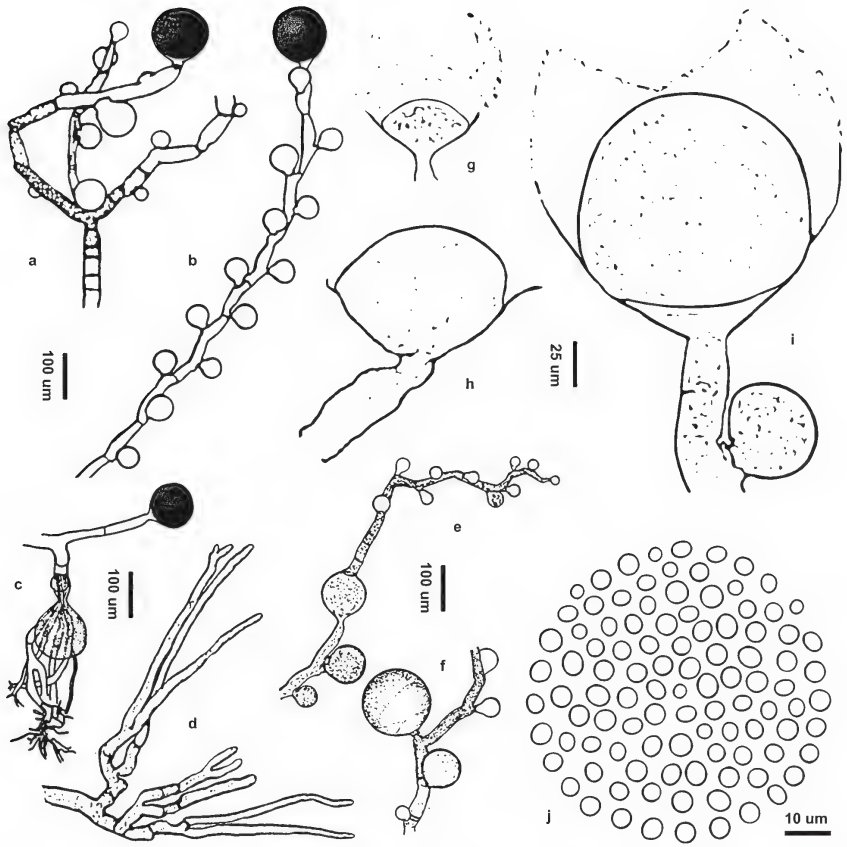

FIGURE 1. *Ambomucor ovalisporus* (CGMCC 3.6806). a, Sporangiophore apex triple branches with an aborted sporangium at the point of separation; b, Single sporangiophore with a fertile top sporangium (in black); c–d, Rhizoids; e–f, Aborted sporangia showing different sizes; g–i, Columellae with distinct collars; j, Sporangiospores. Scale bars: a–f = 100 μm; g–i = 25 um; j = 10 um.

verticillate branches. SEPTA appearing frequently in the hyphae and the main axes of sporangiophores, sporadically in the sporangiophore branches. ABORTED SPORANGIA proliferating repeatedly on the sides of sporangiophores, up to 30 or more, transferring their contents forward and forming a short or long chain of aborted sporangia and terminating in a normal fertile sporangium, pyriform to obovoid and 20–67.5(–83) × 16–58.5(–76) μm, or globose to subglobose and (12.5–)24–85 μm diameter, verrucate, subhyaline. FERTILE SPORANGIA borne terminally on the main axes or the branches of sporangiophores, apophysate, thin-walled, verrucate, globose to subglobose,

26–163.5 µm diameter, dark brown when mature, breaking in larger ones, persistent in smaller ones. Columellae forming in the fertile sporangia only, well developed, verrucate, with a distinct collar, very regular in shape, hyaline; larger ones subglobose and 102.5–186.5 µm diameter, or oblate and 75.5–92(–179) × 55–73.5(–122) µm; smaller ones applanate and 34.5–39.5 × 26.5 µm. Sporangiospores forming in fertile sporangia only, released through breaking or moving backward to the sporangiophores, smooth, mostly oval and 6–12.5 × 4.5–9 µm, sometimes globose or subglobose and (5.5–)6.5–9(–10) µm diameter, hyaline. Chlamydospores absent. Zygospores unknown.

Comments: The maximum growth temperature for *Ambomucor ovalisporus* Am-14 was determined as 27°C (results of two tests). Crosses of *Ambomucor ovalisporus* with *A. clavatus* and *A. seriatoinflatus* var. *seriatoinflatus*, var. *brevior*, and var. *longior* all failed to produce zygospores.

Ambomucor seriatoinflatus* var. *longior X.Y. Liu & R.Y. Zheng, **var. nov.**

MycoBank MB518428 Fig. 2

Differs from other *Ambomucor seriatoinflatus* varieties by its higher colonies, its abundant aerial hyphae, its longer narrower sporangiophore branches, and its later sporulation.

Type: China, Shaanxi Province, Taibai Mountain, Shangbansi Temple, in moss and humus on rock, 11 October 2002, Xue-wei Wang (**Holotype**, HMAS240174-1; **isotypes**, HMAS 240174-2,-3,-4,-5,-6,-7,-8; ex-type cultures, Am-16, CGMCC 3.014179).

Etymology: *longior*, referring to the sporangiophore primary branches being longer than those of *Ambomucor seriatoinflatus* var. *seriatoinflatus*.

Colonies on SMA and PDA floccose, zonate after 3 days, reaching 9 cm diameter in 6–7 days at 18°C, about 3–5 mm high, at first white, then grayish white, at last grayish white on the edge and 'Dark Purplish Gray' (Ridgway LIII) in the middle, reverse 'Olive-Buff' (Ridgway XL). Hyphae branched, nonseptate when young, septate in age, 3–7(–8) µm diameter in substrate, abundant in air and (1–)1.5–5(–8) µm diameter. Stolons absent. Rhizoids scanty, not opposite sporangiophores, arising from various parts of the sporangiophores or from the aborted sporangia. Sporangiophores erect or recumbent, arising directly from substrate or aerial hyphae, forming fertile or aborted sporangia, of three main types: 1) bearing normal fertile sporangia only, 2) aborted sporangia only, or 3) both simultaneously; main axes of the sporangiophores rarely simple, usually branched, 2.9–7.5 µm diameter from substrate, 2–3(–3.5) µm diameter from aerial hyphae; primary branches 1–3, single, in pairs, more often monopodially or pseudoverticillately rebranching, sometimes sympodially and branching no more than three times, (15–)60–543(–1600) µm long, 2–8.5 µm diameter; main axes and branches more or less straight, or subcurved, usually equal or subequal in width throughout, hyaline to pale greenish brown, with

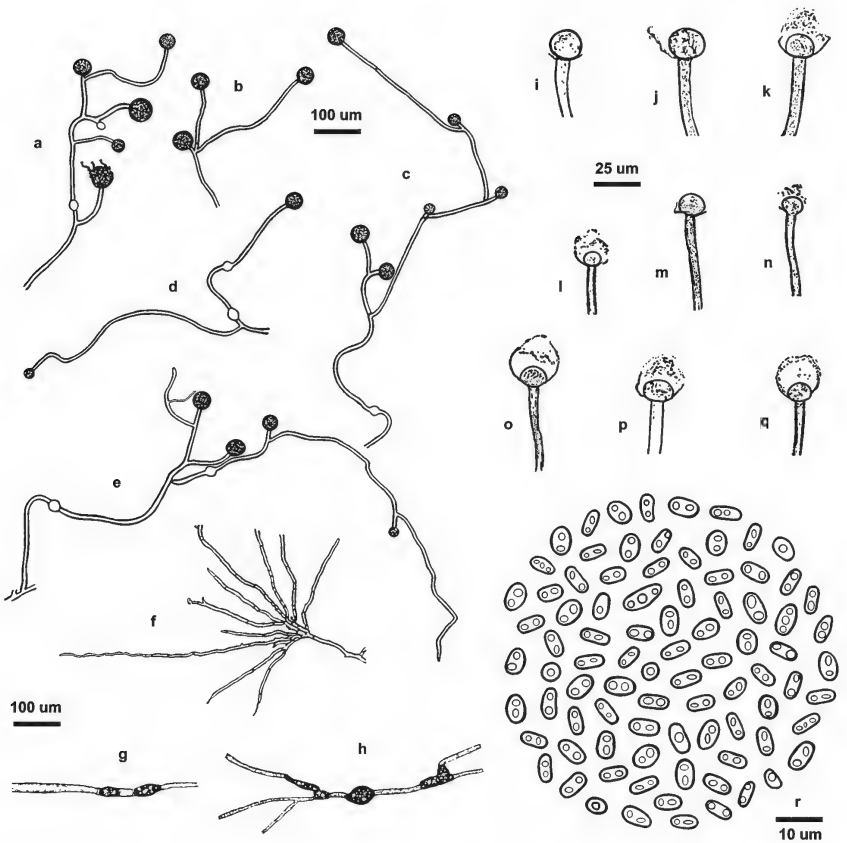

FIGURE 2. *Ambomucor seriatoinflatus* var. *longior* (CGMCC 3.14179). a–e, Upper portions of sporangiophores showing various structures: aborted sporangia (blank) and fertile sporangia (shadowed); f, Rhizoids; g–h, Chlamydospores; i–q, Columellae with distinct collars; r, Sporangiospores. Scale bars: a–h = 100 μm; i–q = 25 μm; r = 10 μm.

or without granular contents, becoming vacuolate in age. SEPTA in main axes and branches of the sporangiophores, usually present at the place of branching, sometimes without definite position. FERTILE SPORANGIA appearing after three days, borne terminally on the main axes or branches of the sporangiophores, globose to slightly depressed globose, (21.5–)24.5–47(–53) μm diameter in larger ones on the sporangiophores from substrate, 7–17 μm diameter in smaller ones on the sporangiophores from aerial hyphae, non-apophysate, breaking or slowly dissolving, thin-walled, dark brown when mature, pale grayish brown in broken pieces of wall. ABORTED SPORANGIA borne terminally on the main

axes and branches of sporangiophores, or repeatedly proliferating terminally or laterally, forming intercalary sporangiophores between two aborted sporangia, no more than three times, transferring their contents forward until a fertile and normal sporangium is formed, non-apophysate or slightly apophysate, ovoid and 10–24 × 7–19 μm, globose and 13–22(–26) μm diameter, granulate when young, vacuolate in age, subhyaline. Columellae formed in fertile sporangia only, well developed, very regular in shape, ovoid to ellipsoid-ovoid, depressed-globose to sub-applanate, 6.5–21 × 5–16.5 μm, hyaline, some with grayish to brownish content, smooth, regularly with a distinct collar. Sporangiospores forming in fertile sporangia, ellipsoid to broadly oblong-ellipsoid and 3.5–7 × 2–3.5 μm, (1–)2-guttulate, hyaline, grayish in mass, smooth, becoming vacuolate in old cultures. Chlamydospores rare, single, or in short chains, subglobose, ovoid or irregular, 5–18.5 × 3–14 μm, hyaline to yellow. Zygospores unknown.

Comments: The maximum growth temperature for *Ambomucor seriatoinflatus* var. *longior* Am-16 was determined as 30°C (results of two tests). Crosses of *Ambomucor seriatoinflatus* var. *longior* with *A. clavatus*, *A. ovalisporus*, and *A. seriatoinflatus* var. *seriatoinflatus* and var. *brevior* all failed to produce zygospores.

Key to the taxa of *Ambomucor*

(revised from Zheng & Liu 2014)

1. Colonies up to 25 mm high; hyphae 21.5–66.5 μm diameter; both fertile and aborted sporangia borne on the same sporangiophore; fertile sporangia apophysate; sporangiospores globose to oval, avacuolate; maximum growth temperature 27°C ..... ***A. ovalisporus***
1. Colonies 1–5 mm high; hyphae 1.5–14 μm diameter; fertile or aborted sporangia or both borne on the same sporangiophore; fertile sporangia non-apophysate; sporangiospores ellipsoid to oblong-ellipsoid, vacuolate; maximum growth temperature no less than 30°C 2
2. Aborted sporangia oval to ellipsoidal; columellae of the fertile sporangia mostly applanate, rarely globose to subglobose; aborted outgrowths frequent, often spirally twisted; maximum growth temperature 33°C ..... ***A. clavatus***
2. Aborted sporangia globose, subglobose to ovoid; columellae of the fertile sporangia usually not as above; aborted outgrowths absent or rare, not spirally twisted when present; maximum growth temperature 30°C 3
3. Aborted sporangia borne in chains of up to 20; aborted outgrowths usually absent and not in a zigzag manner; main axes and branches more or less straight, rarely slightly curved ..... ***A. seriatoinflatus* var. *seriatoinflatus***
3. Aborted sporangia usually not observed exceeding chains of 6; aborted outgrowths when present formed sympodially; main axes and branches subcurved and rarely straight to sub-straight 4

4. Colonies 3–5 mm high; aerial hyphae abundant; fertile sporangia appearing after three days; aborted sporangia globose when diameter is less than 26 μm; columellae less than 21 μm in width ..... ***A. seriatoinflatus* var. *longior***
4. Colonies 1–3 mm high; aerial hyphae scarce; fertile sporangia appearing after one day; aborted sporangia globose, up to 66 μm in diameter; columellae 42 μm or more wide ..... ***A. seriatoinflatus* var. *brevior***

Discussion

Based on the results of studies on several genera of *Mucorales*, such as *Actinomucor* (Zheng & Liu 2005), *Cunninghamella* (Zheng & Chen 2001), *Pilaira* (Zheng & Liu 2009), *Rhizomucor* (Zheng et al. 2009), and *Rhizopus* (Zheng et al. 2007), the range of maximum growth temperatures (MGT) is an important criterion for helping circumscribe genera and distinguish species; however, it is not a decisive characteristic and sometimes there is overlap among taxa. The MGTs of the three *Ambomucor* species (*A. ovalisporus* 27°C; *A. seriatoinflatus* 30°C; *A. clavatus* 33°C) occupy a narrow range that supports their generic affinity, but with significant differences that support the species delimitation.

Acknowledgments

The study was partially supported by the National Natural Science Foundation of China (Nos. 31070019 and 31370068), the Ministry of Science and Technology of China (No. 2012FY111600) and the Foundation of the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-J-6). We greatly appreciate Associate Prof. Xue-wei Wang of this department for collecting and isolating the type strain of *Ambomucor seriatoinflatus* var. *longior*. We also thank Ms. Hong-mei Liu of this research group for preparing the media and plates, helping with isolation, doing mating experiments, and testing the maximum growth temperatures. Finally we thank Dr. Gerald L. Benny (University of Florida, Gainesville, U.S.A.) and Dr. Bo Huang (Anhui Agricultural University, Hefei, P.R. China) for critical comments and presubmission review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.173

Volume 130, pp. 173–179 *January–March 2015*

Perenniporia koreana, a new wood-rotting basidiomycete from South Korea

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Abstract — A new *Perenniporia* species, *P. koreana,* is described based on morphological and molecular data from specimens collected from Gyeonggi-do and Gangwon-do, Korea. It is characterized by annual resupinate basidiocarps that are very pale brown to yellow when dry. Microscopically, it has a dimitic hyphal system with dextrinoid and cyanophilous skeletal hyphae, and its basidiospores are ellipsoid, hyaline, thick-walled, smooth, dextrinoid, cyanophilous, (5.9–)6–7(–7.5) × (3.7–)3.9–5.2(– 5.7) µm.

Key words —phylogeny, *Polyporaceae*, polypore, taxonomy

Introduction

Perenniporia Murrill is a polypore genus of wood-rotting fungi. The characteristics of this genus are: (1) annual to perennial, resupinate to pileate basidiocarps; (2) dimitic to trimitic hyphal system with clamped generative hyphae and non-dextrinoid to strongly dextrinoid or amyloid vegetative hyphae; and (3) usually thick-walled, ellipsoid to truncate, variably dextrinoid or amyloid basidiospores (Gilbertson & Ryvarden 1987; Zhao & Cui 2013a,b). There are about 90 *Perenniporia* species reported in the world and this genus was shown to be polyphyletic when evolutionary relationships were inferred from the representative *Perenniporia* species including the type, *P. medulla-panis* (Jacq.) Donk (Robledo et al. 2009; Zhao et al. 2013; Zhao & Cui 2013a,b).

In Korea, nine *Perenniporia* species have been recorded: *P. fraxinea* (Bull.) Ryvarden, *P. fraxinophila* (Peck) Ryvarden, *P. medulla-panis*, *P. minutissima* (Yasuda) T. Hatt. & Ryvarden, *P. ochroleuca* (Berk.) Ryvarden, *P. ohiensis* (Berk.) Ryvarden, *P. subacida* (Peck) Donk, *P. tephropora* (Mont.) Ryvarden, and *P. truncatospora* (Lloyd) Ryvarden (Lee & Jung 2005; Park & Lee 2011). During research on indigenous fungi in South Korea, we found eight specimens that represent *Perenniporia* but had characteristics that deviated from the previously reported species. Based on morphological and molecular analyses, we describe them as a new species.

Materials & methods

Collection and morphological examination

Eight *Perenniporia* specimens collected from Gyeonggi-do and Gangwon-do in Korea were used in this study. We examined them macro- and microscopically according to Jang et al. (2013). Spores were measured in Melzer's reagent and 5% of the measurements from both ends are presented in parentheses (Zhao & Cui 2013a). Color codes follow Munsell (2009). The studied specimens have been deposited at the Herbarium of National Institute of Biological Resources, Korea (KB).

Phylogenetic analysis

Genomic DNA was extracted from the specimens according to Jang et al. (2012). PCR reactions were primed with ITS1F (Gardes & Bruns 1993) / ITS4 (White et al. 1990) for internal transcribed spacer (ITS) region and with LR0R/LR7 (Vilgalys & Hester 1990) for nuclear large subunit ribosomal DNA (nLSU) region according to Jang et al. (2012). DNA was sequenced by Macrogen DNA sequencing service (Seoul, Korea), and the sequences were deposited in GenBank, NCBI. The ITS and nLSU sequences of closely related species were retrieved from GenBank. The sequences were aligned with MAFFT 7.130 (Katoh & Standley 2013) using the L-INS-i method for each dataset. For Bayesian analysis, the best-fit model for each dataset was calculated by MrModeltest 2.3 (Nylander 2004) under AIC criterion. The two datasets were combined and the selected models were applied. The 50% majority rule consensus tree was calculated by MrBayes 3.2 (Ronquist et al. 2012) according to Jang et al. (2013).

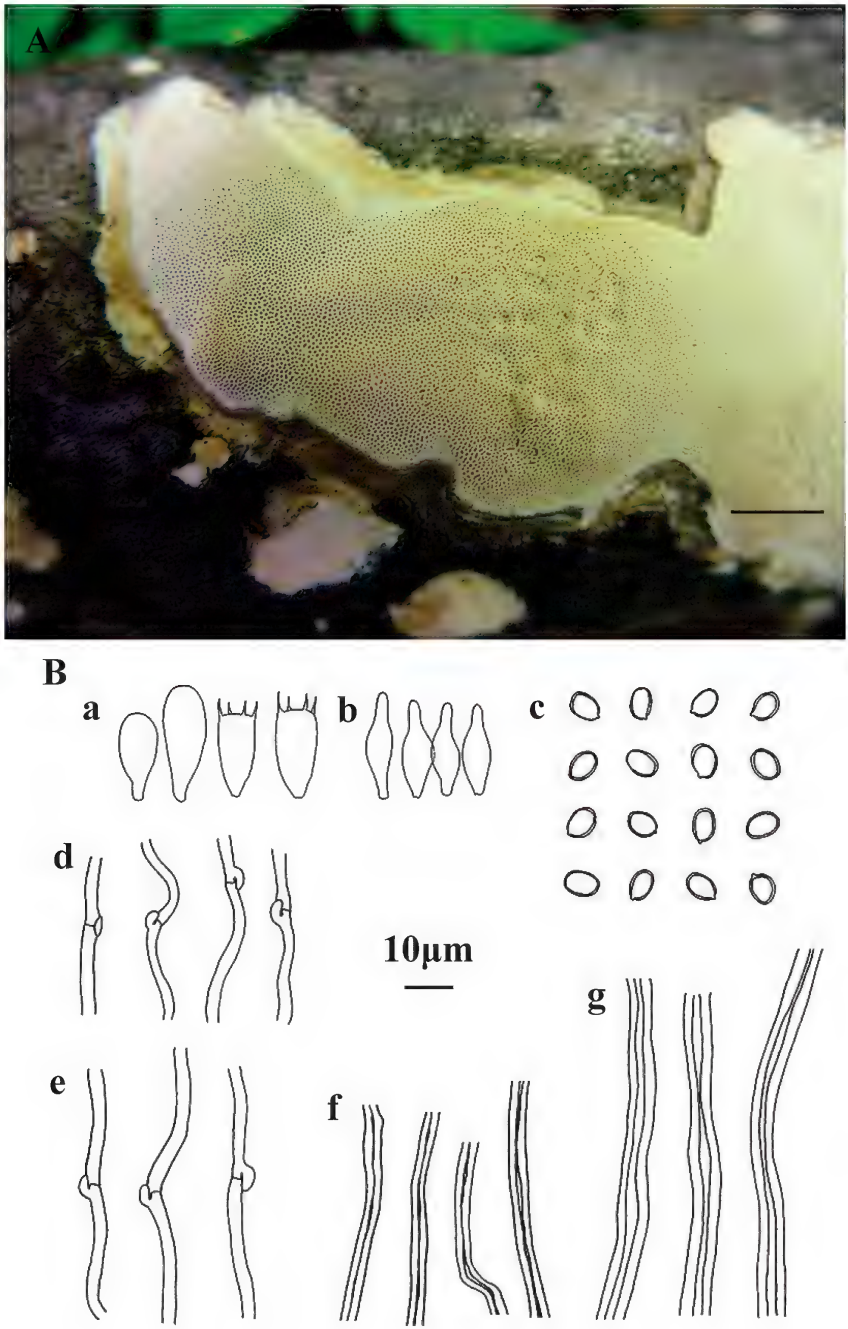
Taxonomy

Perenniporia koreana Y. Jang & J.J. Kim, **sp. nov.** Fig. 1

MycoBank MB 807626

Differs from other *Perenniporia* species by its annual resupinate basidiocarps with grayish orange pore surface, lack of rhizomorphs in most specimens, a dimitic hyphal system with dextrinoid skeletal hyphae, and ellipsoid non-truncate dextrinoid basidiospores.

Fig. 1. *Perenniporia koreana* (KB NIBRFG107080, holotype). A. Basidiocarp. Scale bar = 1 cm. B. Microscopic features: a, basidioles and basidia; b, cystidioles; c, basidiospores; d, generative hyphae from trama; e, generative hyphae from context; f, skeletal hyphae from trama; g, skeletal hyphae from context.


A
B
a
b
c
d
e
f
g
10μm

Type: Korea. Kyeonggi-do, Dongducheon-si, Mt. Soyo, 37°56′44″N 127°04′08″E, on the branch of *Quercus* sp., 17 May 2008, Jae-Jin Kim KUC20080517-15 (**Holotype**, KB NIBRFG107080; GenBank KJ156301, KJ156309).

Etymology: The locality (Korea) of the type specimen.

Basidiocarps annual, resupinate, adnate, ca. 8.5 cm or more in longest dimension, ca. 4 cm in widest dimension. Sterile margin felty, orange gray (10YR8/2), up to 1 cm wide, usually without rhizomorphs (present only in KB NIBRFG107396). Pores round to angular, 5–6 per mm; dissepiments thin, entire; pore surface grayish orange (10YR8/3-8/4) to brownish orange (10YR7/6-7/8) in dried condition. Context corky, cream to buff, up to 0.5 mm thick. Tubes corky, concolorous with pore surface, up to 2 mm thick.

Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae dextrinoid, cyanophilous.

Context generative hyphae hyaline, thin-walled, 1.5–3 µm in diameter; skeletal hyphae dominant, hyaline, thick-walled, with a distinct lumen, interwoven, 3.5–6 µm in diameter.

Tubes generative hyphae hyaline, thin-walled, 1.5–2.5 µm in diameter; skeletal hyphae dominant, hyaline, thick-walled, with a narrow lumen to subsolid, rarely branched, interwoven, 1.5–4.5 µm in diameter. Cystidia absent, fusoid cystidioles present, 19.5–23 × 5–6.5 µm; basidia barrel-shaped, 4 sterigmate, 21–23 × 7.5–9 µm.

Basidiospores ellipsoid, hyaline, thick-walled, smooth, dextrinoid, cyanophilous, (5.9–)6–7(–7.5) × (3.7–)3.9–5.2(–5.7) µm, L = 6.56, W = 4.45 (n = 120/4).

Ecology & distribution — Korea, on hardwoods causing white rot.

Additional specimens examined: **KOREA, Kyeonggi-do,** Uijeongbu-si, Mt. Dobong, on fallen wood branch, 30 October, 2009, Yeongseon Jang KUC20091030-32 (KB NIBRFG113564; GenBank KJ156305, KJ156313); Yongin-si, Mt. Gwanggyo, 37°21′05″N 127°03′14″E, on hardwood, 14 August 2012, Yeongseon Jang KUC20120814-17 (KB NIBRFG125415; GenBank KJ156306, KJ156314); Yangju-si, Mt. Goryeong, on wood, 11 July 2009, Yeongseon Jang KUC20090711-70 (KB NIBRFG113613; GenBank KJ156304, KJ156312); Dongducheon-si, Mt. Soyo, 37°56′44″N 127°04′08″E, on the branch of hardwood, 17 May 2008, Jae-Jin Kim KUC20080517-02 (KB NIBRFG107071; GenBank KJ156300, KJ156308); **Gangwon-do,** Heongseong-gun, Mt. Chiak, on wood, 4 April 2009, Young Woon Lim KUC20090404-27 (KB NIBRFG113243; GenBank KJ156303, KJ156311); 37°20′16″N 128°03′27″E, on hardwood, 2 October 2008, Jae-Jin Kim KUC20081002J-02 (KB NIBRFG107396; GenBank KJ156302, KJ156310); Wonju-si, Mt Chiak, 37°21′43″N 128°02′36″E, on the branch of *Quercus* sp., 10 May 2008, Jae-Jin Kim KUC20080510-01 (KB NIBRFG107760; GenBank KJ156299, KJ156307).

Phylogeny

The combined ITS+nLSU dataset contained 56 taxa and 2016 characters, of which the ITS dataset was 667 characters and nLSU was 1349 characters. By the

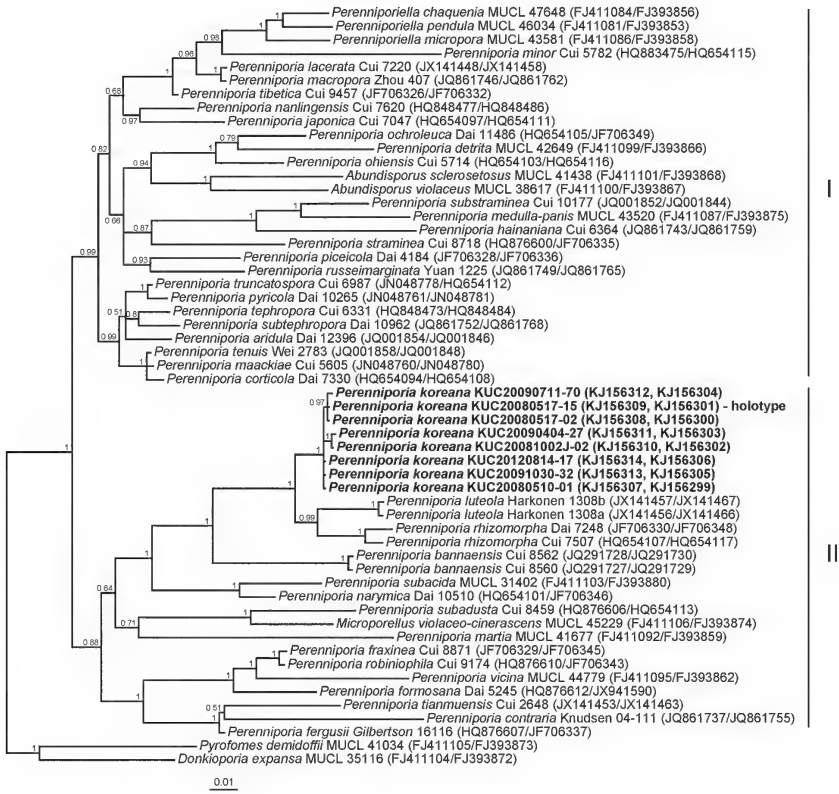

Perenniporiella chaquenia MUCL 47648 (FJ411084/FJ393856)
Perenniporiella pendula MUCL 46034 (FJ411081/FJ393853)
Perenniporiella micropora MUCL 43581 (FJ411086/FJ393858)
Perenniporia minor Cui 5782 (HQ883475/HQ654115)
Perenniporia lacerata Cui 7220 (JX141448/JX141458)
Perenniporia macropora Zhou 407 (JQ861746/JQ861762)
Perenniporia tibetica Cui 9457 (JF706326/JF706332)
Perenniporia nanlingensis Cui 7620 (HQ848477/HQ848486)
Perenniporia japonica Cui 7047 (HQ654097/HQ654111)
Perenniporia ochroleuca Dai 11486 (HQ654105/JF706349)
Perenniporia detrita MUCL 42649 (FJ411099/FJ393866)
Perenniporia ohiensis Cui 5714 (HQ654103/HQ654116)
Abundisporus sclerosetosus MUCL 41438 (FJ411101/FJ393868)
Abundisporus violaceus MUCL 38617 (FJ411100/FJ393867)
Perenniporia substraminea Cui 10177 (JQ001852/JQ001844)
Perenniporia medulla-panis MUCL 43520 (FJ411087/FJ393875)
Perenniporia hainaniana Cui 6364 (JQ861743/JQ861759)
Perenniporia straminea Cui 8718 (HQ876600/JF706335)
Perenniporia piceicola Dai 4184 (JF706328/JF706336)
Perenniporia russeimarginata Yuan 1225 (JQ861749/JQ861765)
Perenniporia truncatospora Cui 6987 (JN048778/HQ654112)
Perenniporia pyricola Dai 10265 (JN048761/JN048781)
Perenniporia tephropora Cui 6331 (HQ848473/HQ848484)
Perenniporia subtephropora Dai 10962 (JQ861752/JQ861768)
Perenniporia aridula Dai 12396 (JQ001854/JQ001846)
Perenniporia tenuis Wei 2783 (JQ001858/JQ001848)
Perenniporia maackiae Cui 5605 (JN048760/JN048780)
Perenniporia corticola Dai 7330 (HQ654094/HQ654108)
Perenniporia koreana KUC20090711-70 (KJ156312, KJ156304)
Perenniporia koreana KUC20080517-15 (KJ156309, KJ156301) - holotype
Perenniporia koreana KUC20080517-02 (KJ156308, KJ156300)
Perenniporia koreana KUC20090404-27 (KJ156311, KJ156303)
Perenniporia koreana KUC20081002J-02 (KJ156310, KJ156302)
Perenniporia koreana KUC20120814-17 (KJ156314, KJ156306)
Perenniporia koreana KUC20091030-32 (KJ156313, KJ156305)
Perenniporia koreana KUC20080510-01 (KJ156307, KJ156299)
Perenniporia luteola Harkonen 1308b (JX141457/JX141467)
Perenniporia luteola Harkonen 1308a (JX141456/JX141466)
Perenniporia rhizomorpha Dai 7248 (JF706330/JF706348)
Perenniporia rhizomorpha Cui 7507 (HQ654107/HQ654117)
Perenniporia bannaensis Cui 8562 (JQ291728/JQ291730)
Perenniporia bannaensis Cui 8560 (JQ291727/JQ291729)
Perenniporia subacida MUCL 31402 (FJ411103/FJ393880)
Perenniporia narymica Dai 10510 (HQ654101/JF706346)
Perenniporia subadusta Cui 8459 (HQ876606/HQ654113)
Microporellus violaceo-cinerascens MUCL 45229 (FJ411106/FJ393874)
Perenniporia martia MUCL 41677 (FJ411092/FJ393859)
Perenniporia fraxinea Cui 8871 (JF706329/JF706345)
Perenniporia robiniophila Cui 9174 (HQ876610/JF706343)
Perenniporia vicina MUCL 44779 (FJ411095/FJ393862)
Perenniporia formosana Dai 5245 (HQ876612/JX941590)
Perenniporia tianmuensis Cui 2648 (JX141453/JX141463)
Perenniporia contraria Knudsen 04-111 (JQ861737/JQ861755)
Perenniporia fergusii Gilbertson 16116 (HQ876607/JF706337)
Pyrofomes demidoffii MUCL 41034 (FJ411105/FJ393873)
Donkioporia expansa MUCL 35116 (FJ411104/FJ393872)
0.01
I
II

Fig. 2. 50% majority consensus tree of *Perenniporia koreana* and allied species inferred from combined ITS+nLSU region sequences. Posterior probabilities over 0.5 are shown above branches. GenBank accession numbers are presented in parentheses (ITS/nLSU). *Perenniporia koreana* is indicated by bold type.

model selection, GTR+I+G model was chosen for both ITS and nLSU datasets. *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar (MUCL 41034) and *Donkioporia expansa* (Desm.) Kotl. & Pouzar (MUCL 35116) were used as outgroup (Zhao & Cui 2013a,b; Zhao et al. 2013). The phylogenetic tree (Fig. 2) provides the sequence information for the included taxa. Our analysis supported two main groups, I and II. Group I, which contained *Perenniporia* species with typically truncate basidiospores, included *P. medulla-panis* (the type species) as well as *Abundisporus sclerosetosus* Decock & Laurence, *A. violaceus* (Wakef.) Ryvarden, *Perenniporiella chaquenia* Robledo & Decock, *P. pendula* Decock & Ryvarden, and *P. micropora* (Ryvarden) Decock & Ryvarden. Group II included

Perenniporia species with non-truncate basidiospores (except for *P. fergusii* Gilb. & Ryvarden with ellipsoid to slightly truncate basidiospores; Gilbertson & Ryvarden 1987), and *Microporellus violaceocinerascens* (Petch) A. David & Rajchenb. *Perenniporia koreana* was in group II and monophyletic with high posterior probability value (1.0 p.p.). Its sister taxa were *P. luteola* B.K. Cui & C.L. Zhao and *P. rhizomorpha* B.K. Cui et al., and these three taxa clustered with *P. bannaensis* B.K. Cui & C.L. Zhao, *P. subacida*, and *P. narymica* (Pilát) Pouzar in a clade with high support (1.0 p.p.).

Discussion

Morphologically, *Perenniporia koreana* is very similar to its sister taxa, *P. luteola* and *P. rhizomorpha,* which are also characterized by a resupinate basidiocarp, yellowish pore surface, dextrinoid skeletal hyphae, and dextrinoid non-truncate basidiospores. However, *P. luteola* differs by its perennial habit and slightly wider basidiospores (6.1–6.9 × 5.1–5.4 µm; Zhao & Cui 2013a), and *P. rhizomorpha* differs by having typical rhizomorphs, slightly longer basidiospores (5.3–6.5 × 4.1–5.2 µm), contextual skeletal hyphae encrusted with fine crystals and lacking cystidia and cystidioles (Cui et al. 2007).

Perenniporia tenuis (Schwein.) Ryvarden and *P. bannaensis* are similar to *P. koreana* in their annual resupinate basidiocarps, yellowish pore surface, dimitic hyphal system with dextrinoid skeletal hyphae, fusoid cystidioles, and dextrinoid basidiospores. However, *P. tenuis* differs in its larger pores (3–5/mm) and small ellipsoid to truncate basidiospores (4.5–6 × 3.5–4.5 µm; Ryvarden & Gilbertson 1994), while *P. bannaensis* differs in its small pores (6–8/mm), entire to lacerate dissepiments, and shorter basidiospores (5.2–6 × 4–4.6 µm; Zhao et al. 2013).

Phylogenetically (Fig. 2), the species relationships in our analysis differ substantially from those of Zhao et al. (2013) and Zhao & Cui (2013a,b). Our analysis clustered *Perenniporia luteola, P. rhizomorpha,* and *P. bannaensis* in group II, a relationship also observed when *P. koreana* was excluded from the phylogenetic analysis (not shown). In contrast, *P. rhizomorpha* and *P. bannaensis* were monophyletic with *P. medulla-panis* in Zhao et al. (2013); and *P. luteola, P. rhizomorpha,* and *P. bannaensis* clustered with *P. medulla-panis* in Zhao & Cui (2013a,b). Further research with additional loci would help to resolve the species relationships of *Perenniporia*.

In conclusion, we propose *Perenniporia koreana* as a new species with only minute morphological distinction but with distinct genetic differences.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390) and was supported by the Indigenous Species Survey

and Investigation project from the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. We are much obliged to Dr. Bao-Kai Cui and Dr. Tsutomu Hattori for their valuable suggestions on the manuscript.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.181

Volume 130, pp. 181–190 *January–March 2015*

New records of corticolous myxomycetes from Turkey

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Abstract– Eight myxomycete species from the Marmara region are newly recorded for Turkey: *Amaurochaete tubulina, Comatricha longipila, Diderma cinereum, D. umbilicatum, Didymium lenticulare, Physarum serpula, Stemonitis inconspicua,* and *Symphytocarpus confluens.*

Keywords– *Quercus* bark, moist chamber technique

Introduction

The Marmara region of Turkey is located in the Euro-Siberian and the Mediterranean phytogeographical regions of Turkey. The western part of the region is subjected to Mediterranean climate and is covered with pine forests and shrubs, whereas the eastern and northern parts have a continental climate and are rich in oak forests (Atalay 2002).

Turkey is an important world distribution area for *Quercus* diversity (Günal 1997). There are 18 species of oak trees in Turkey (Seçmen et al. 1995), of which 11 occur in the Marmara region.

The first myxomycetes for Turkey were reported by Härkönen & Uotila (1983) and Härkönen (1987). Although the number of myxomycete species worldwide is about 1000 (Lado 2001), the reported numbers for Turkey are relatively low, around 232 (Sesli & Denchev 2014). With this paper we contribute further knowledge on the myxomycetes in the country by presenting eight species collected on bark of *Quercus* trees in the Marmara region, all new records for Turkey.

Materials & methods

Field excursions were carried out in a randomised manner during June and October of 2005–2008. All material was collected from the bark of *Quercus* species in different

provinces of Turkey's Marmara region. Myxomycete fructifications were obtained from moist chamber cultures in the laboratory as described in Stephenson & Stempen (1994). The cultures were moistened with distilled water and kept under diffuse sunlight within a temperature range of 22–25°C.

The cultures were examined under a dissecting microscope every day for a 4-week period. Whenever developing fructifications were found, the bark pieces on which they were found were removed from the Petri dish, allowed to dry slowly, and then placed in herbarium boxes. The same chambers were then rewetted for another 4-week period and examined as before. The specimens are stored in the authors' personal collections in the Department of Biology, Uludağ University, Bursa, Turkey. Species have been identified according to Castillo et al. (1997), Farr (1976, 1981), Ing (1999), Keller & Brooks (1977), Lado & Pando (1997), Martin & Alexopoulos (1969), Mitchell (2013), Nannenga-Bremekamp (1991), Stephenson & Stempen (1994), and Thind (1997). Nomenclature follows Lado (2001).

Species recorded

Amaurochaete tubulina (Alb. & Schwein) T. Macbr. PLATE 1

Sporocarps aethaliate. Aethalia pulvinate, somewhat depressed. Sporothecae covered by a thin, translucent cortex. Hypothallus thin, shining, prominent. Capillitium branched, irregularly sprouting out from the base, branching and anastomosing freely. Spore mass black. Spores pale olivaceous, 12–15 µm diam., finely warted.

SPECIMEN EXAMINED – **TURKEY**, **BILECIK/MERKEZ**; Karadere–Sütlük road, 40°05′35.3″N 29°49′51.9″E, alt. 977 m, on bark of *Quercus frainetto* in oak forest, 22.7.2007, Oran 303–1.

COMMENTS—*Amaurochaete* is a typical genus in the order *Stemonitales* characterized by a dendroid capillitium. Previously only a single species, *A. atra* (Alb. & Schwein.) Rostaf., has been reported from Turkey (Sesli & Denchev 2014). An examination of published records of *A. tubulina* indicates that this species is frequently isolated from conifer bark, whereas our specimen was recovered from *Quercus* bark in a moist chamber culture. Our specimen appears to represent the first aethaliate structure of an *Amaurochaete* obtained from a moist chamber culture.

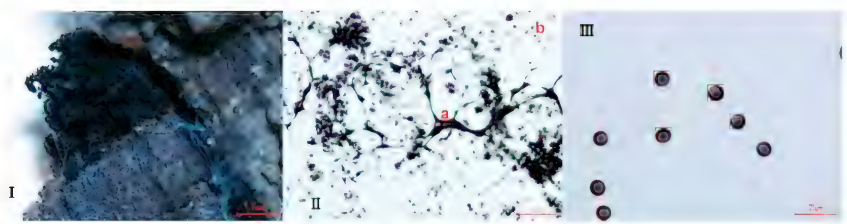

PLATE 1. *Amaurochaete tubulina*. I– aethalium; II– a. capillitium, b. spores; III– spores.

Amaurochaete tubulina somewhat resembles *A. atra* and *A. comata* G. Lister & Brândză. It differs from *A. atra,* which is clearly distinguished by an early fugacious aethelial wall, in its capillitial structure that widens in the axils and is connected to the columella but not connected to neighbouring sporangia. *Amaurochaete tubulina* is distinguished from *A. comata* by its sparsely anastomosing free ends, spiny capillitia, and spores that are less strongly warted on one side.

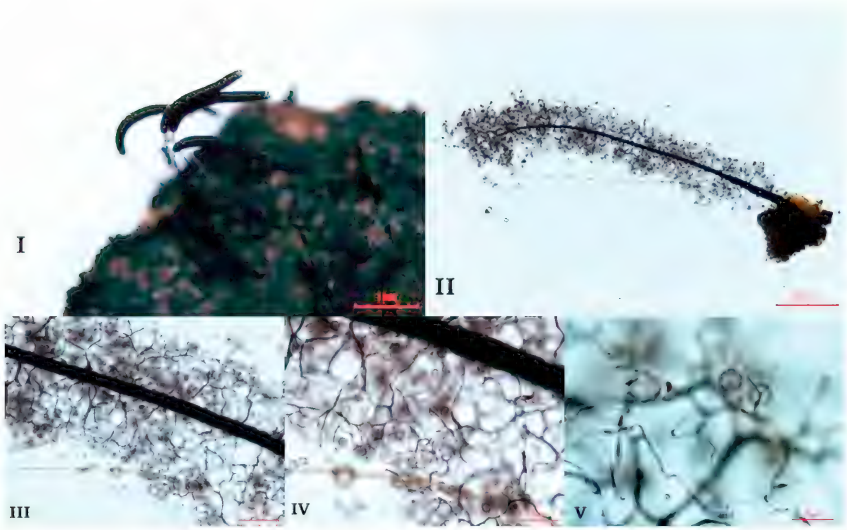

Plate 2. *Comatricha longipila*. I– sporocarps; II– a. hypothallus; b. columella; III– a. columella; b. capillitium; c. spinose capillitial ends; IV– a. columella, b. capillitium, c. spore; d. capillitial ends; V– a. capillitium, b. reticulate spores.

Comatricha longipila Nann.-Bremek. Plate 2

Sporocarps in small groups or solitary, 1.5–2.0 mm tall, nearly erect. Hypothallus discoid or continuous under the group, brown and inconspicuous. Stalk up to 33% of the total height, with a net of fibres at the base, often opaque above. Sporotheca cylindrical or obovoid, 0.3–0.6 mm diam., brown. Peridium usually completely fugacious but sometimes persistent as a collar around the stalk apex. Columella merging into the capillitium just below the apex of the sporotheca. Capillitium primary branches perpendicular to the columella, hardly anastamosing, dichotomously branched with many, rather straight, long, free ends at the periphery which are sometimes slightly swollen. Spores pale red-brown, 6.0–7.0 μm diam., finely warted.

Specimen examined – **TURKEY, Kirklareli/Merkez**; Üsküp–Beypınarı road 2 km, 41°45′05.7″N 27°26′32.4″E, alt. 403 m, on bark of a solitary *Quercus pubescens* tree in agricultural land, 15.6.2006, Oran 91–2.

Comments—Our specimen, as was the case for the type specimen, appeared in a moist chamber culture prepared with a bark sample from *Quercus*. The peridium occasionally remains as a permanent collar around the stalk. As noted by Nannenga-Bremekamp (1991), "*C. longipila* is characterized by the lax internal capillitium, with long free ends at the periphery and its small spores." However, Ing (1999) indicated that its small spores, lack of an outer capillitial network, and long free ends separate *Comatrica longipila* from small specimens of *C. laxa* Rostaf.

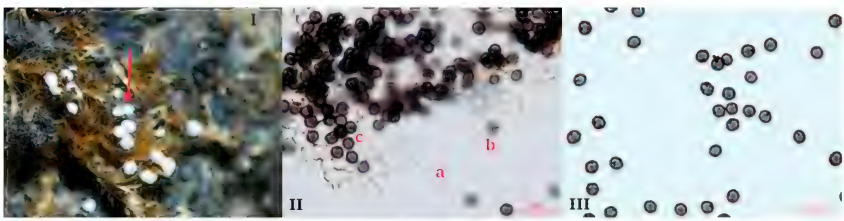

Plate 3. *Diderma cinereum*. I– individual sporocarps;
II– a. capillitium, b. peridium, c. spores; III– verruculose spores.

Diderma cinereum Morgan Plate3

Sporocarps sessile, gregarious, subglobose, somewhat depressed, 0.3–0.5 mm diam., pearl-grey. Hypothallus inconspicuous. Peridium single, thin, smooth, crustose. Capillitium of slender, dark threads, sparsely branched, easily detached from the columella. Spore-mass black. Spores dark violet-grey, 9–11 µm diam., verruculose.

Specimen examined – **TURKEY, Balikesir/Merkez**; Konakpınar–Söpköy road 5 km, 39°25′00.8″N 27°50′06.5″E, alt. 331 m, on bark of *Quercus cerris*, 28.7.2006, Oran 242–2.

Comments—There are various reports of the presence of a single or double peridium in this species, but our specimen clearly has a single peridium. Ing (1999) observed that *D. cinereum* is similar to *D. spumarioides* (Fr.) Fr. but differs in having a single peridium, deciduous capillitium, and dark grey spores.

Diderma umbilicatum Pers. Plate 4

Sporocarps short-stalked or sessile, pearl-grey. Peridium double, the outer layer calcareous or occasionally limeless, adhering closely to the membranous inner layer. Dehiscence irregular, leaving a ragged cup which shows white on the inside. Columella prominent, pale, subglobose, smooth or rugose. Stalk stout, reddish-brown, covered with white lime. Spores dark brown in mass, purple brown in transmitted light, densely verruculose, 10–12 µm diam.

Specimens examined – **TURKEY, Çanakkale/Yenice**; around Gönen dam, 39°57′41.1″N 27°25′52.2″E, alt. 191 m, on bark of *Quercus cerris* in composite forest,

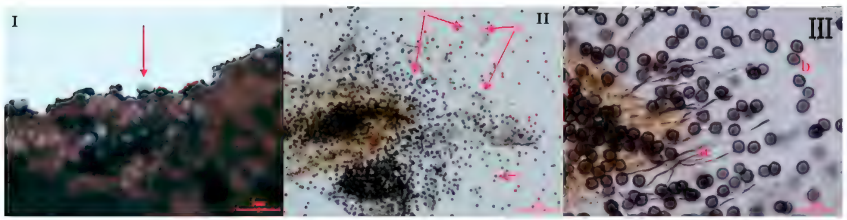

Plate 4. *Diderma umbilicatum*. I– individual sporocarp;
II– a. peridial fragments, b. capillitium, c. spores; III– a. capillitium, b. spores.

29.7.2006, Oran 253–1; **Sakarya/Geyve**; Bozcağız–Koru road, 4 km to Koru village, 40°31′33″N 30°26′49″E, alt. 350 m, on bark of *Quercus virgiliana* in oak–beech forest, 7.10.2007, Oran 266–1.

Comments—*Diderma umbilicatum* is included in *Diderma* subg. *Leangium* (Link) Macbr. and has often been considered a variety of *D. radiatum* (L.) Morgan (Nannenga-Bremekamp 1991). Ing (1999) suggested that *D. umbilicatum* resembles both *D. montanum* (Meyl.) Meyl. and *D. radiatum*, especially with respect to peridial characteristics. Hence, it was considered to be synonymous with *D. radiatum* until quite recently. However, it is actually quite different, having a number of features (e.g., irregular dehiscence, non-cartilaginous peridium, pale columella, stout stalk) that are not present in *D. radiatum*. Under a hand lens, it may resemble *D. montanum*, from which it can be distinguished by its thicker and reddish short stalk, large columella, and usually larger spores. Ing (1999) noted that its large pale sporangia, conspicuous pale columella, and thick stalk easily separate *D. umbilicatum* from other *Diderma* taxa.

Didymium lenticulare K.S. Thind & T.N. Lakh. Plate 5

Sporocarps stipitate, up to 2 mm tall, scattered to loosely gregarious. Hypothallus dark brown, rotate. Stalk 1–1.8 mm long, erect, grooved, tapered, dark brown below, lighter and translucent above, scantily charged with lime crystals, particularly at the base. Sporotheca erect or nodding, strongly discoid, deeply umbilicate at the base, white, 0.5–0.8 mm diam. Peridium membranous, hyaline, densely covered with lime deposits which form very irregular ridges and plates. Columella absent. Capillitium of stout, subhyaline threads, sparingly branched and anastomosed and with bead-like thickenings. Spore-mass black. Spores deep violaceous brown by transmitted light, globose, distinctly verrucose, also marked by conspicuous clusters of darker and bigger warts, 9–11 μm diam.

Specimen examined – **TURKEY, Kirklareli/Pinarhisar**; Çayırdere–Akören road 2 km, 41°42′19.3″N 27°31′15.7″E, alt. 269 m, on bark of *Quercus frainetto* in an oak stand, 25.7.2006, Oran 179–2.

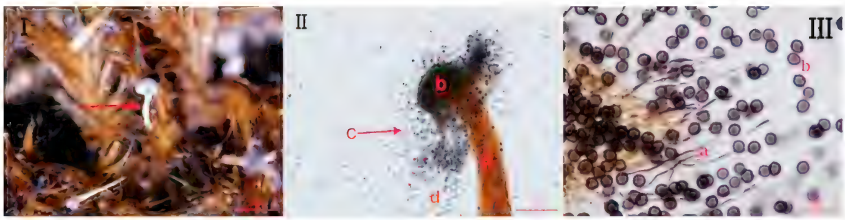

Plate 5. *Didymium lenticulare*. I– individual sporocarp;
II– a. stipe, b. pseudocolumella, c. capillitial threads; d. spores;
III– a. crusted lime, b. capillitium, c. spores.

Comments—*Didymium lenticulare* very closely resembles *D. clavus* (Alb. & Schwein.) Rabenh. but differs in a number of features, including its hyaline, crustose peridium with thick ridges, longer stipe, more dispersed lime crystals (especially in the lower region), lack of a columella, and spores that are larger, lighter in color, and distinctly verrucose (Thind 1977).

This species also resembles *D. squamulosum* (Alb. & Schwein.) Fr. in its possession of a hyaline peridium and similar spores but differs in its densely thick calcareous deposits on the peridium, strongly discoid sporotheca, and lack of a columella. In addition, its stipe is much longer and darker than that typically observed in *D. squamulosum*. The stipe of *D. lenticulare* is non-calcareous, although it is sprinkled with lime crystals.

The stipe also differs from that of *D. iridis* (Ditmar) Fr., which has strongly discoid sporangia that are deeply umbilicate below. The extraordinarily small lime crystals of *D. lenticulare* also serve to distinguish it from allied species (Thind 1977).

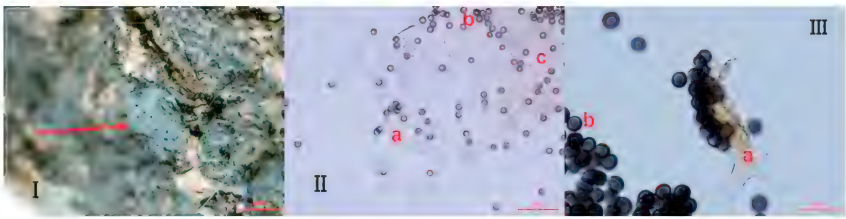

Plate 6. *Physarum serpula*. I– plasmodiocarp;
II– a. lime node, b. spores, c. peridium; III– a. lime node, b. spores.

Physarum serpula Morgan Plate 6

Plasmodiocarpous, forming lines, rings, or a simple network, 0.2–0.4 mm wide, sometimes fusing laterally to form a broad fruiting surface, often interspersed with globose sporocarps, dull yellow or ochraceous, sessile. Hypothallus diffuse. Peridium single, thin, of closely encrusted lime globules,

without calcareous scales, fragile, membranous, persistent. Capillitium dense, calcareous, the nodes numerous, large, angular, branching, pale yellow or whitish, connected by short, hyaline threads or broad limy strands and then almost badhamioid. Spore-mass dull black. Spores dark brown, verruculose, with a paler and smoother area on one side, 10–13 µm diam.

Specimen examined – **TURKEY, Bursa/Nilüfer**; around Alaaddinbey village, 40°12′10.7″N 28°53′45.2″E, alt. 78 m, on bark of a solitary *Quercus robur* tree in agricultural land, 16.9.2007, Oran 312–2.

Comments—*Physarum serpula* was treated by Ing (1999) as part of a trio with *P. auriscalpium* Cooke and *P. decipiens* M.A. Curtis, and Martin & Alexopoulos (1969) observed that these three morphologically similar species were often confused with one another. *Physarum serpula* is frequently confused with *P. auriscalpium,* which differs by having a peridium dotted with prominent glossy lime scales rather than the peridium evenly impregnated with lime in *P. serpula.* Ing (1999) observed that *P. decipiens,* which is more sporangiate than *P. auriscalpium* or *P. serpula,* has a more badhamioid capillitium.

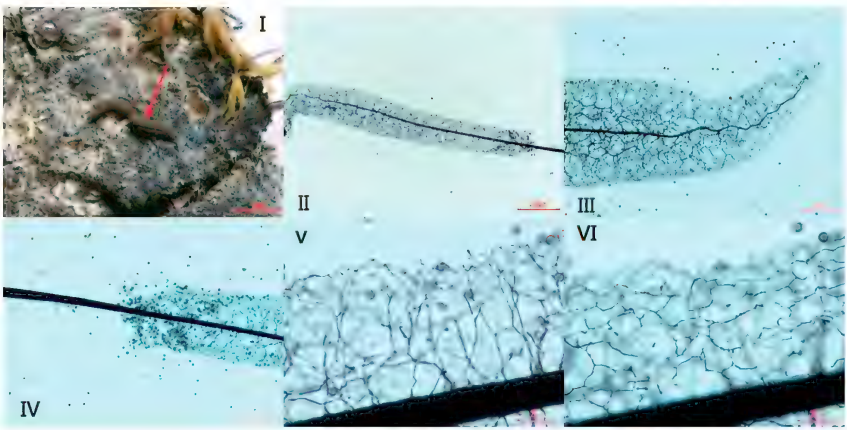

Plate 7. *Stemonitis inconspicua.* I) sporocarps; II– individual sporocarp; III– apical end of sporotheca; IV– basal end of sporotheca; V– inner net of capillitium; VI– surface net.

Stemonitis inconspicua Nann.-Bremek. Plate 7

Sporocarps clustered on a common hypothallus, sometimes gregarious, total height 2–8 mm. Hypothallus thin, silvery, red-brown by light microscope. Stalk approximately 0.5 mm long, thin, black, opaque, shining. Sporothecae brown, blunt cylindrical, 1.5–2.5 mm tall. Peridium fugacious, except sometimes at the base, where red-brown fragments show a structure similar to that of the spores. Columella tapered, dissipating just below the apex. Capillitium forming approximately 3 meshes on the radius, consisting of slender threads variously

widened at the junctions, becoming extremely fine towards the periphery, the surface net slender, irregular, fragmentary, with spiny free ends, dissipating towards the apex. Spore mass dark brown. Spores pale red-brown, prominently banded-reticulate with 2–3 meshes across the hemisphere, 8–9 µm diam. excluding the bands, which form a border approximately 0.5 µm high in optical section.

Specimens examined – **TURKEY**, **Bilecik/Merkez**; Karadere–Sütlük road, 3 km to Sütlük, 40°05′35.3″N 29°49′51.9″E, alt. 977 m, on bark of *Quercus frainetto* in an oak stand, 22.7.2007, Oran 3–1; **Çanakkale/Merkez**; Çan–Çanakkale road, Kocalar turnout, 40°02′03.7″N 26°46′57.1″E, alt. 469 m, on bark of *Quercus frainetto* in an oak stand, 6.7.2005, Oran 303–4.

Comments—*Stemonitis inconspicua* can be distinguished from the similar *S. foliicola* Ing by its small size and banded-reticulate spores (Ing 1999).

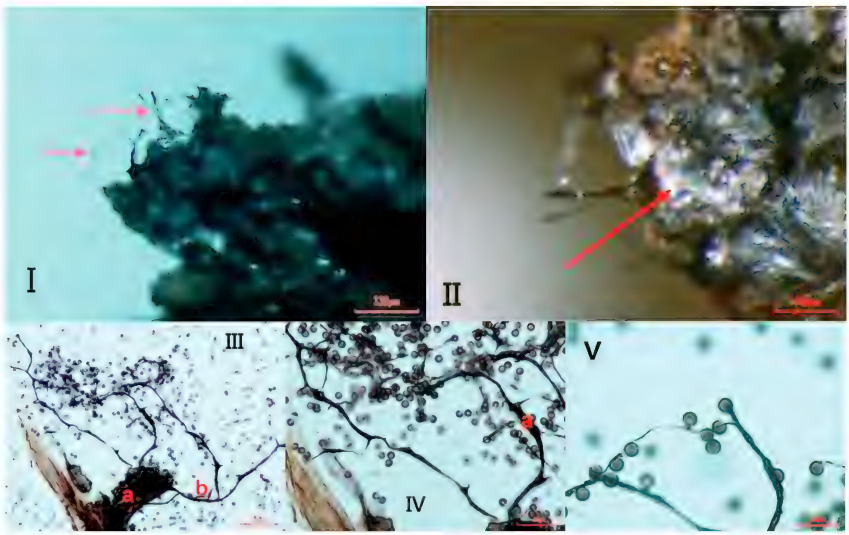

Plate 8. *Symphytocarpus confluens*. I– pseudoaethalium; II– silver coloured hypothallus; III– a. hypothallus and stipe, b. capillitium; IV– a. enlargements on capillitium, b. spores; V) spores.

Symphytocarpus confluens (Cooke & Ellis) Ing & Nann.-Bremek. Plate 8

Pseudoaethalium 2–3 mm tall and 0.5–4 cm diam., deep black. Sporothecae approximately 0.5 mm diam. Hypothallus silvery. Peridium fugacious except for a number of more or less rounded plates, smooth and connected to the capillitium. Columella absent or, if present, opaque, dissipating below the apex. Capillitium a wide-meshed reticulum of thick, dark purple threads with small axillary membranes, looped at the periphery. Spores dark purple-brown in transmitted light, (10–)11–13 µm diam., distinctly warted.

Specimen examined – **TURKEY, Kirklareli/Merkez**; Dereköy–Kula road, around Geçitağzı village, 41°57′34.9″N 27°21′43.4″E, alt. 624 m, on bark of *Quercus petraea*, 16.6.2006, Oran 110–1.

Comments—*Symphytocarpus confluens,* which is close to *S. impexus* Ing & Nann.-Bremek, differs in its shorter, more elongated, deep black sporangia, which occasionally possess an opaque columella that dissipates below the apex of the sporangium (although it is not uncommon for the columella to be absent). Hence, the sporangia of *S. confluens* are much deeper black in color and have much larger spores that are at least 10 (usually ≥12) μm in diameter (Nannenga-Bremekamp 1991).

Discussion

Bark from *Quercus* trees sampled from 11 cities and six provinces in the Marmara region were the source of our new records for Turkey: Balikesir province (*Diderma cinereum*), Bilecik province (*Amaurochaete tubulina, S. inconspicua*), Çanakkale province (*D. umbilicatum, S. inconspicua*), Bursa province, (*P. serpula*), Kırklareli province (*C. longipila, D. lenticulare, S. confluens*), and Sakarya province (*D. umbilicatum*). As the distribution shows, two species (*Didymium umbilicatum, Stemonitis inconspicua*) were recovered from two different provinces.

The character of the tree bark structure is an important factor for myxomycete distribution and productivity. Härkönen et al. (2004) concluded that a smooth basic bark has a low water holding capacity and does not support a high diversity of myxomycetes, bryophytes, and lichens. McHugh (1998) noted that individual *Quercus* species and *Quercus* forests are the most productive sources for myxomycetes. Bark from oak trees offers a suitable structure for sheltering myxomycete spores. Stephenson (1989) noted that the considerable variation in bark structure offers good potential microhabitats for corticolous myxomycetes. High water holding capacities may benefit corticolous myxomycetes directly as their active plasmodia are highly dependent on the availability of liquid water.

Acknowledgements

We thank Prof. S.L. Stephenson (University of Arkansas) and D.W. Mitchell for their invaluable suggestions to our manuscript. We offer also sincere thanks for the effort and meticulous attention to the nomenclature provided Dr. S.R. Pennycook. We thank also AJE for article editing process.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.191

Volume 130, pp. 191–196 *January–March 2015*

A new species of *Lophodermium* with variously branched paraphyses

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Abstract — *Lophodermium heterocladum*, a new fungus developing on leaves of *Rhododendron cavaleriei*, was collected from the Mount Sanqingshan National Park of Jiangxi Province, China. Description, illustration, and comments are provided for this taxon. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — foliicolous fungus, taxonomy, fungal diversity, *Ericaceae*

Introduction

During research on leaf-inhabiting fungi on members of the *Ericaceae* in the Mount Sanqingshan National Park of China, one specimen of the ascomycete family *Rhytismataceae* was obtained. Examination of that material and comparison with previously described species (Lin et al. 2001a,b) led to the conclusion that the specimen represents a new species of *Lophodermium* Chevall. Fifty-seven *Lophodermium* species have previously been reported from China (Hou et al. 2009, Luo et al. 2010, Shi et al. 2010, Chen et al. 2012, Lin et al. 2012, Wang et al. 2012, Chen et al. 2013, Gao et al. 2013, Wang et al. 2014, Lu et al. 2015).

Materials & methods

The conidiomata and ascomata were examined macroscopically with a dissecting microscope at 10–50× magnification. After rehydration in water for ca 10 min, fruiting bodies were cut into 10–15 µm thick sections using a freezing microtome. The sections were mounted in water, KOH solution, Melzer's reagent, cotton blue in water, or lactophenol-cotton blue and examined microscopically. The colors of various structures

were observed in water. Gelatinous sheaths surrounding ascospores and paraphyses were examined in 0.1% (w/v) lactophenol-cotton blue. Measurements were made using material in 5% KOH and from more than 30 paraphyses, asci, ascospores, conidiogenous cells, and conidia for each specimen. Point and line integrated illustrations of external status and internal structures of fruiting bodies were drawn using a microscopic drawing device. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Lophodermium heterocladum H.L. Gu & Y.R. Lin, **sp. nov.** FIGS 1–8

MycoBank MB 808959

Differs from *Lophodermium implicatum* by its variably shaped paraphyses that branch 1–2(–3) times, the presence of periphysoids and hyphal bridges, and a well-developed subhymenium forming a strongly concave bowl shape.

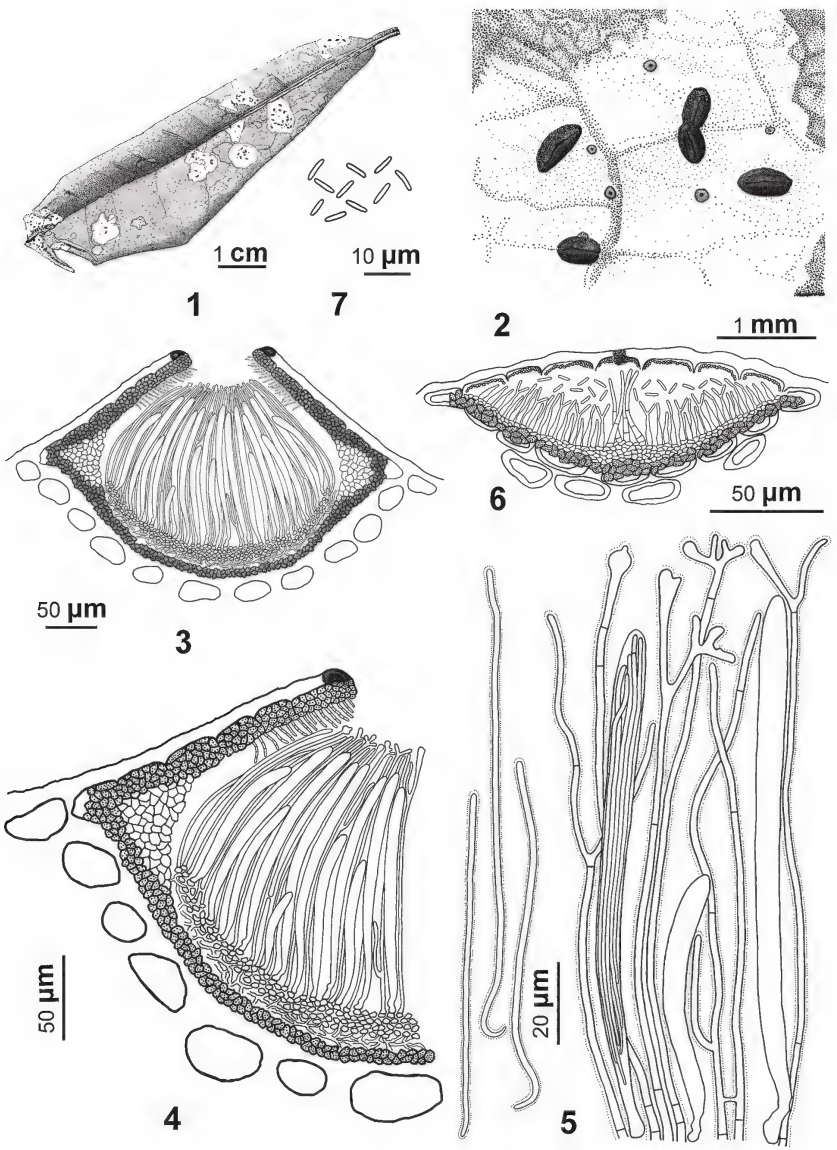
TYPE: China, Jiangxi, Mount Sanqingshan National Park, Huaiyushan, alt. ca 600 m, on fallen leaves of *Rhododendron cavaleriei* H. Lév. (*Ericaceae*), 18 August 2012, Y.R. Lin, F. Zhou & L. Zhang 2604 (**Holotype**, AAUF 68712).

ETYMOLOGY: *heterocladum*, referring to variably branched paraphyses of the fungus.

ZONE LINES absent.

CONIDIOMATA developing on upper side of leaves scattered in pale sandy brown, subcircular or irregular, pale areas with an obvious edge. In surface view, conidiomata 100–170 µm diam., rounded or subrounded, black-brown in the centre and the perimeter line of the conidioma, grey-brown elsewhere, slightly raising the substratum surface, discharging spores through an inconspicuous central ostiole. In median vertical section, conidiomata intraepidermal, lenticular. UPPER WALL extremely poorly developed, composed of a small amount of tiny angular cells. BASAL WALL dark brown, consisting of 1–3 layers of 3–5 µm diam., thick-walled angular cells. SUBCONIDIOGENOUS LAYER 4–10 µm thick, comprised of colorless, thin-walled angular cells 1–3 µm diam. TRICHOGYNES 32–38 × 2.5–3.2 µm, cylindrical, tapering towards the rounded apex, 1–3-septate in the lower half. CONIDIOPHORES not observed. CONIDIOGENOUS CELLS 10–14 × 1.8–2.4 µm, ampullaceous, colorless, usually proliferating sympodially and percurrently. CONIDIA 4–6 × 0.8–1 µm, hyaline, aseptate, cylindrical, sometimes slightly curved, obtuse or round at the ends, smooth-walled.

ASCOMATA in similar positions to conidiomata on the host, scattered to clustered, occasionally coalescent. In surface view, ascomata 350–680 × 170–360 µm, elliptical, ends round or obtuse, black, slightly shiny, with a clearly marked outline, moderately raising the substratum surface, but slightly sunken near the split, opening by a single longitudinal split which extends almost to the ascomatal edge. Lips absent. In median vertical section, ascomata intraepidermal. COVERING STROMA 12–20 µm thick, mainly composed of



Figs 1–7. *Lophodermium heterocladum* (holotype) on *Rhododendron cavaleriei.* 1. Habit on a leaf. 2. Detail of fruiting bodies. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci and ascospores. 6. Conidioma in vertical section. 7. Conidia.

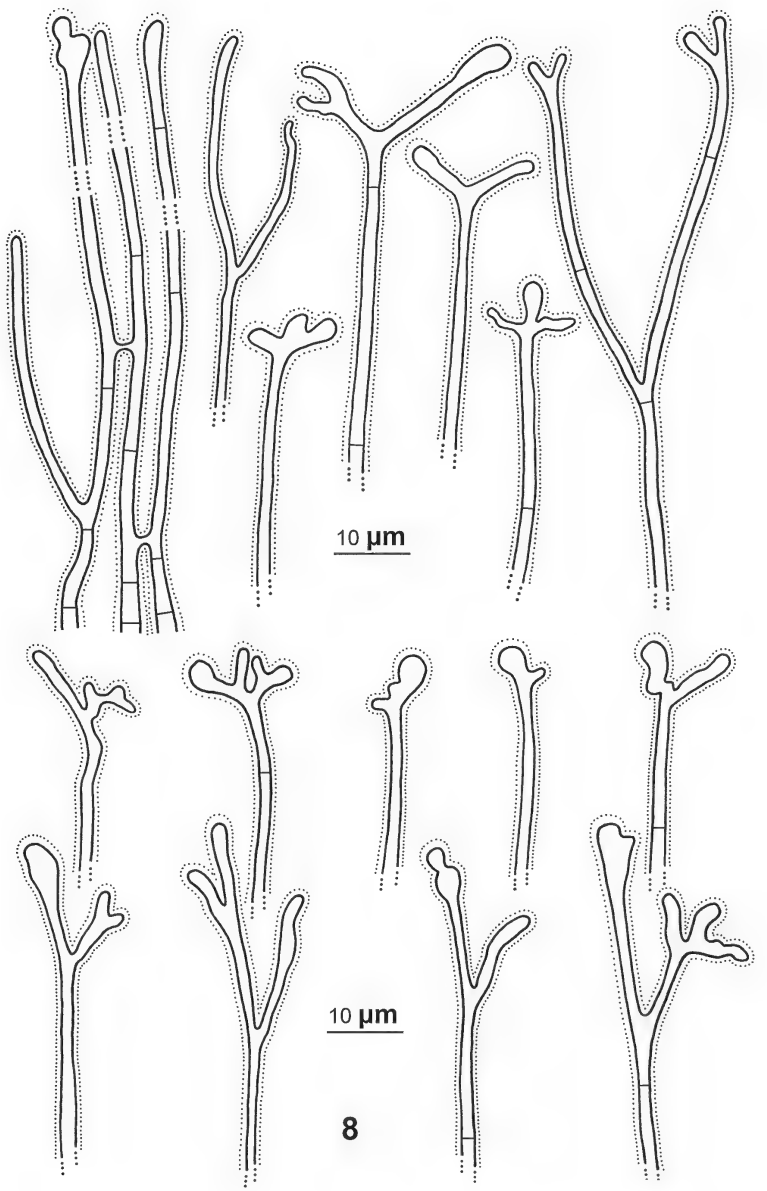


Fig. 8. *Lophodermium heterocladum* (holotype): variously branched paraphyses.

textura angularis with dark brown to black, thick-walled cells 3–5 µm diam., connecting to the basal stroma. There is a 6–8 µm thick mulch which comprises strongly carbonized tissue with no obvious cellular structure outside the top of the covering stroma. Periphysoids lining the inner face of the covering stroma, 5–14 × 1–1.5 µm, cylindrical, not swelling at the apex, hyaline, straight or slightly curved, 0–4-septate. Basal stroma dark-brown, consisting of 1–3 layers of thick-walled angular cells 3–7 µm diam. The triangular space between the covering stroma and basal stroma is filled with hyaline, thin-walled angular cells 4–9 µm diam. Subhymenium well developed, 15–22 µm thick, strongly concave and forming a bowl shape, composed of colorless textura angularis and intricata. Paraphyses 130–155 × 1.5–2.2 µm, filiform, hyaline, thinly septate, mostly gradually or abruptly swollen to 4.2–5.8 µm at the apex, branching 1–2(–3) times often near the top and in the middle or occasionally in the positions not far from the base, sometimes with hyphal bridges near the base between adjacent paraphyses, surrounded by a ca 0.5 µm thick gelatinous matrix. Asci ripening sequentially, 110–140 × 5.5–7.2 µm, cylindrical, apex round, short-stalked, thin-walled, J–, 8-spored. Ascospores arranged in a fascicle, 85–120 × 1.2–1.6 µm, filiform, hyaline, aseptate, straight or slightly curved, apex round or obtuse, hardly tapered towards the round or acute base, with a thin gelatinous sheath.

Host species, habitat, & distribution: Producing conidiomata and ascomata on fallen leaves of *Rhododendron cavaleriei*. Known only from the type locality, Jiangxi Province, China.

Comments — *Lophodermium heterocladum* is very similar to *L. implicatum* Y.R. Lin & Z.S. Xu on *Rhododendron maculiferum* subsp. *anhweiense* (E.H. Wilson) D.F. Chamb. in the way ascomata and conidiomata are embedded and in ascal shape and size. However, *L. implicatum* has ascomata on both surfaces of the leaf, dark brown zone lines, the corner between the covering stroma and basal stroma consisting of a very small amount of colorless, thin-walled angular cells, paraphyses branched near the apex and entangled to form a light yellow-brown epithecium 8–12 µm thick, subhymenium composed of colorless textura porrecta, and lacks periphysoids and hyphal bridges (Lin et al. 2001a).

Lophodermium rufum Y.R. Lin & K. Li on *Rhododendron maculiferum* subsp. *anhweiense* is easily distinguished from the new species by its much larger (620–1040 × 430–580 µm) subcuticular ascomata, well-developed rufous lips, covering and basal stroma consisting of textura angularis-epidermoidea, wider (8–10.5 µm) asci, shorter and wider (54–86 × 1.5–2 µm) ascospores containing many granules, unbranched paraphyses slightly gradually swollen at the apex (Lin et al. 2001b).

Acknowledgments

The authors are grateful to Dr J.E. Taylor and Dr M. Ye for pre-submission reviews leading to the improvement of our manuscript, and to Mr F. Zhou and Ms L. Zhang for the field investigations. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31170019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.197

Volume 130, pp. 197–205 *January–March 2015*

Bertia hainanensis sp. nov. (*Coronophorales*) from southern China

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Abstract — A new species of *Bertia* collected in southern China is described and illustrated. *Bertia hainanensis* is characterized by a combination of such features as non-ostiolate ascomata with a roughened tuberculate surface, 1-septate cylindrical-geniculate ascospores, and filiform paraphyses. It is most similar to *B. tropicalis*, from which it differs by its narrower ascospores and its filiform paraphyses. The distinctive features of the ascomatal surface were examined with a scanning electron microscope. The validity of the family *Bertiaceae* is discussed.

Key words — *Ascomycota*, coronophoralean fungi, SEM images, taxonomy

Introduction

In 2012, the second author collected a specimen of *Bertia* De Not. from the Bawangling National Nature Reserve (Hainan Province, China), and we consider it to represent a species new to science. As in our previous studies on several other coronophoralean fungi (Vasilyeva et al. 2010, 2012a, 2013), we prepared images of fruiting bodies using a scanning electron microscope (SEM).

There are only a few records of *Bertia* species from China. *Bertia macrospora* Sacc. and *B. moriformis* (Tode) De Not. have been reported from Gansu, Jiangsu,

Jilin, and Yunnan provinces (Teng 1996); *B. sinensis* J.C. Krug & Corlett was described from Yunnan (Krug & Corlett 1988) and *B. biseptata* Sivan. & W.H. Hsieh from Taiwan (Hsieh et al. 1995).

Materials & methods

The type specimen of the new species is deposited in the Fungarium of the Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan, China (FCATAS). Material was prepared for SEM examination in accordance with Vasilyeva et al. (2012a). Light micrographs of asci and ascospores were obtained using a Leica DM 4500B microscope, and a Zeiss EVO 40 scanning electron microscope was used to examine surface details of the ascomata.

Taxonomy

Bertia hainanensis Lar.N. Vassiljeva, H.X. Ma, Chernyshev & S.L. Stephenson, **sp. nov.** FIG. 1

MycoBank MB 857516

Differs from *Bertia tropicalis* by its narrower ascospores and its filiform paraphyses.

TYPE: China, Hainan Province, Bawangling National Nature Reserve, on dead branches of an unidentified tree, 5 November 2012, Hai-Xia Ma (**Holotype**: FCATAS 300).

ETYMOLOGY: refers to the province of China where the fungus was collected.

ASCOMATA superficial, mostly aggregated, turbinate, with a thick sterile base, collapsing collabent when dry, black, 640–820 µm diam., with a roughened, tuberculate surface, tubercules 50–75 × 35–50 µm.; walls 40–60 µm thick, consisting of 3–4 layers of angular cells 7–11 × 6–8 µm; Munk pores present, 0.8–1 µm diam. ASCI elongated-clavate, 8-spored, long-stipitate, with a kind of apical ring ca. 2 µm, in the spore-bearing portions (72–)80–100 × 14–18 µm, stalks 100–120 µm, oozing from ascomata in fascicles intermingled with numerous filiform paraphyses. ASCOSPORES overlapping biseriate, cylindrical, basal one-third curved, geniculate, 1-septate, hyaline, 24–28 × 4.8–5.5 µm.

NOTES: The most similar species described in the literature appears to be *Bertia tropicalis* Huhndorf et al., which differs by its wider ascospores (5-9.5 µm) and the presence of inflated paraphyses (Huhndorf et al. 2004). *Bertia tropicalis* is known from Costa Rica, French Guiana, Jamaica, Panama, and the United States (Puerto Rico), all localities that are consistent with a Caribbean center of fungal biodiversity. As such, *B. tropicalis* could be considered as a vicarious species with respect to *B. hainanensis*, which might be expected to occur in other countries within the southeastern Asian center of fungal diversity (cf. Vasilyeva et al. 2012b). *Bertia hainanensis* differs from both *B. tropicalis* and *B. sinensis* in its more narrow ascospores but shares with *B. tropicalis* the collapsing collabent ascomata in contrast to the elongated and non-collapsing ascomata that characterize *B. sinensis*.

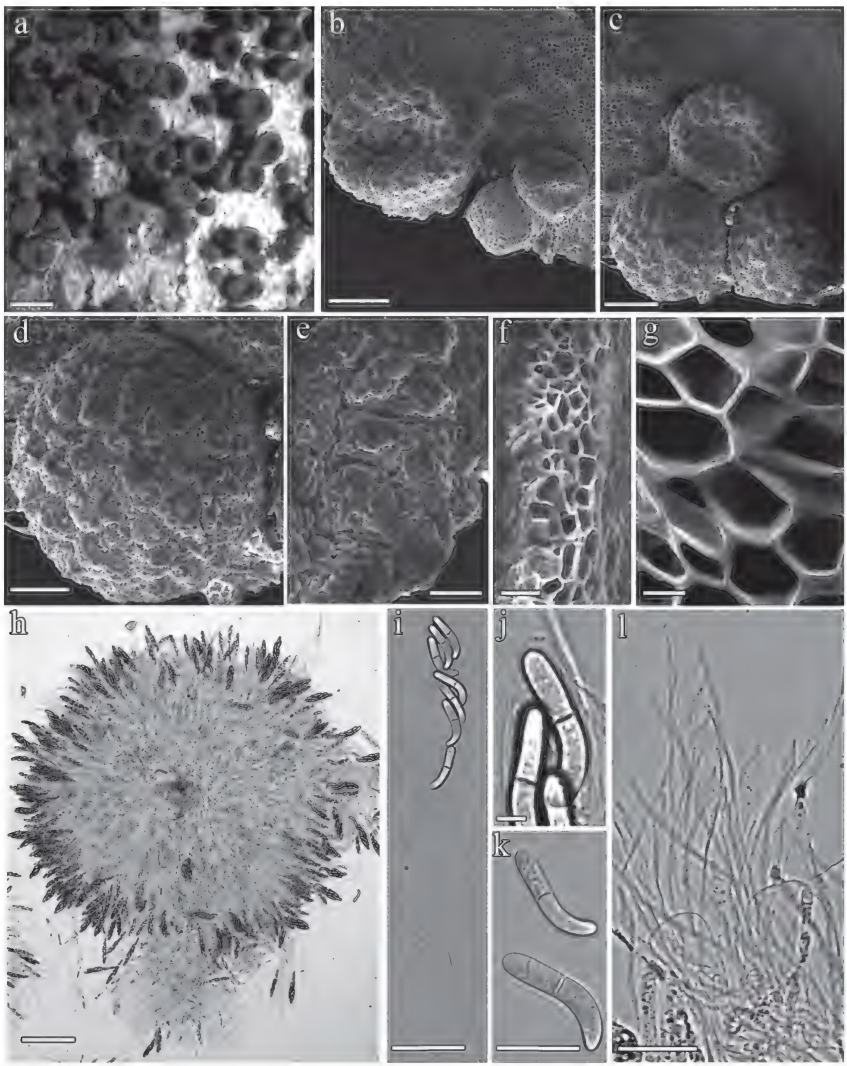

FIG.1. *Bertia hainanensis* (holotype, FCATAS 300): a–d. Ascomata; e. Ascomatal surface; f, g. Cross section through a lateral wall; h. Fascicle of asci and paraphyses; i. Ascus with ascospores; j. Ascal apex showing a tiny apical ring; k. Ascospores; l. Paraphyses. Scale bars: a = 0.5 mm; b, c = 200 µm; d, h = 100 µm; e, i = 50 µm; f, k, l = 20 µm; g, j = 5 µm.

Other members of the relatively small group of *Bertia* species known from southeastern Asia are *B. fructicola* Henn. and *B. oxyspora* (Penz. & Sacc.) Höhn. from Indonesia (Hennings 1894a; Penzig & Saccardo 1897), *B. novoguineensis*

Henn. from the northeast of the island of New Guinea (Hennings 1894b), and *B. tessellata* Petch and *B. turbinata* Petch from Sri Lanka (Petch 1922). Almost all of these have wider ascospores than *B. hainanensis*, with *B. oxyspora* also characterized by longer ascospores (≤50 µm). Only *B. tessellata* has an ascospore width (4.5–5.0 µm) that is similar to that of *B. hainanensis*, but ascospores of *B. tessellata* are pale fuscous and 3-septate.

Discussion: some remarks on the *Bertiaceae*

Both the genus *Bertia* and the family *Bertiaceae* Smyk are poorly investigated, and their current composition is uncertain. Smyk (1981) proposed the family without referring to its distinguishing characters, although she cited the cytological studies of ascomata and asci by several French mycologists (Luc 1952, Chadefaud 1954, Parguey-Leduc 1977) as warranting segregation of *Bertia* into a new family. As circumscribed by Smyk (1981), the new family included three genera (*Bertia*, *Calyculosphaeria* Fitzp., and *Rostrocoronophora* Munk), but *Calyculosphaeria* species are now considered to belong to *Nitschkia* G.H. Otth ex P. Karst., while *Rostrocoronophora* has been transferred to a different order (*Diaporthales*) as a synonym of *Gnomonia* Ces. & De Not. (Bolay 1972). One might wonder just which characters could support the family *Bertiaceae* sensu Smyk.

A search for characters in the French literature cited by Smyk (1981) also has not revealed the possible criteria for distinguishing the family *Bertiaceae*. All three publications compared *B. moriformis* with species from other—sometimes very distantly related—groups, and such a comparison has surely provided a number of differences, but if a new family is segregated within the order *Coronophorales*, as in the case of the study by Smyk (1981), it should be based upon differences among the members of this order. Luc (1952) compared *B. moriformis* and *Systremma natans* (Tode) Theiss. & Syd. [= *Dothidea sambuci* (Pers.) Fr., *Dothideales*] and listed several characters between them (e.g., asci with double walls thickened at the apex versus asci with single and thin walls without a thickening at the apex), but these very same characters distinguish the roughly outlined groups *Ascohymeniales* and *Ascoloculares* (Nannfeldt 1932). So, although those characters properly separate *B. moriformis* and *S. natans* into higher taxonomic groups, they cannot serve as a basis for delimiting the family *Bertiaceae* since different hierarchical levels of a taxonomic system are characterized by their own characters. In addition, Luc (1952) compared the asci of *B. moriformis* with those found in *Chaetomium* Kuntze and *Melanospora* Corda (although without referring to particular species) and concluded that *B. moriformis* might be an 'intermediate' taxon that occupies a position between the order *Dothideales* and the class *Haerangiomycetes* as created (Falck & Falck 1947) for *Melanospora* and *Ophiostoma* Syd. & P. Syd. However, this clearly

represents an example of unfairly judging the "phylogenetic" position of *B. moriformis* without properly analyzing numerous groups, and such a peculiar comparison cannot be considered as the basis for creating the *Bertiaceae*.

Chadefaud (1954) compared *B. moriformis* with another dothidealean species, *Dothidella ulmi* (C.-J. Duval) G. Winter. His conclusions were similar to those by Luc (1952), and *B. moriformis* was suggested as being separate from the *Dothideales*, but one cannot estimate the level of differences with only two species under comparison. Parguey-Leduc (1977) discussed different modes of ascus formation along with the structure of ascus wall and apical ring in pyrenomycetous fungi. She mentioned *B. moriformis* only once, indicating that its dangeardium (i.e., the ascus initial) is two-celled (a rare condition for the pyrenomycetes). However, the same dangeardium was also observed in *Cordyceps militaris* (L.) Fr., *Epichloe typhina* (Pers.) Tul & C. Tul., and *Mycosphaerella bolleana* B.B. Higgins, so that feature might also represent a case of parallelism in different fungal groups — a relatively frequent phenomenon. Again, one cannot find any reason to warrant the *Bertiaceae* as a separate family.

Quite recently Huhndorf et al. (2004) accepted the *Bertiacea*e because two species (*B. moriformis* and *B. tropicalis*) form a well-supported clade on the molecular tree of the *Coronophorales*. However, two circumstances should be taken into consideration. First, a taxon cannot exist without delineating the differences that distinguish it from other taxa at the same hierarchical level. Second, the level of 'clades' (familial, subfamilial, etc.) is unknown without an estimation of differences between tentatively segregated groups. As has been pointed out, taxa occupying the same taxonomic level are characterized by a state combination of the same characters (Vasilyeva & Stephenson 2012, 2013). Still more recently, Mugambi & Huhndorf (2010) provided the necessary distinguishing characters for the *Bertiaceae*, but once again these appear to be unsatisfactory. Members of this family are supposed to have ascomata that are mostly tuberculate (if smooth, then the ascospores are uniformly brown) and ascospores longer than 15 μm. However, ascospore size cannot be used for discriminating among families, since this character is universally used for species delimitation. As for the presence of tubercules, these structures characterize many members of the *Coronophorales* and differ only in size and shape (Vasilyeva et al., 2010, 2012a, 2013). Moreover, the absence of tubercules is also permitted in the *Bertiaceae*, so this character clearly cannot be a criterion for the differentiation of the family.

In general, the simple accumulation of differences for the circumscription of a group is often useless, since it is not the number of characters but the weight of each character that actually matters. Very often, a single difference might be of great value. As pointed out elsewhere (Vasilyeva & Stephenson 2012), the weight of a character is its position in the taxonomic hierarchy, and this weight

depends upon the frequency at which a character is used to delimit groups at a certain level. Judging from the characters that have been proposed for the segregation of the *Bertiaceae*, we have no choice but to abandon this family until more substantial characters are found. The position of *Bertia* within *Nitschkiaceae* Nannf. seems acceptable for the time being, although there is a question about the differences between *Bertia* and *Nitschkia*.

For example, *Nitschkia macrospora* Teng has rather large and rough tubercules on the stromatal surface (Vasilyeva et al. 2010), a condition almost comparable with that found in *B. latispora* (Corlett & J.C. Krug) Lar.N. Vassiljeva (Fig. 2). Moreover, *N. macrospora* ascospores are large (30–45 µm long), so this species appears to fit the concept of *Bertia*, at least sensu Mugambi & Huhndorf (2010). On the other hand, *B. orbis* Mugambi & Huhndorf (Mugambi & Huhndorf 2010: Fig. 5r) has ascospores that are typical for *Nitschkia*. The clustering of *B. orbis* with other *Bertia* species apart from *Nitschkia* species in the molecular tree (Mugambi & Huhndorf 2010: Fig. 1) is not sufficient evidence supporting their independence. In general, the dichotomous structure of molecular trees strongly influences the composition of clades.

To illustrate this situation, we can use one of the ascomycetous families. If one keeps the combinatorial variation of fungi in mind and attempts to construct a dichotomous tree of the genera of the family *Gnomoniaceae*, the type genus *Gnomonia* will fall in the same clade as *Apiognomonia*, *Gnomoniella*, and *Ophiognomonia* when ascomatal type is used to define this clade. In contrast, *Gnomonia* will appear in the same clade as *Chalcosphaeria*, *Melanopelta*, and *Plagiostoma* when ascospore type found in each genus is considered (Vasilyeva & Stephenson 2010). Molecular data might support both arrangements (if they are correlated with defining features) or point towards new ones, but it is highly probable that all genera in the *Gnomoniaceae* comprise a network of taxa instead of reflecting a cladistic structure, with several characters that are of equal value defining genera in the combinatorial space. The same situation might be observed among the genera that comprise the *Coronophorales*, and we have already pointed out the partial combinatorial variation within this order (Vasilyeva et al. 2013). The same conclusion could be reached by considering the key to genera provided by Mugambi & Huhndorf (2010), where several differences (e.g., asci 8-spored vs. multispored and ascomata spinulose vs. not spinulose) are repetitive in the delimitation of genera. The choice of any such equal-leveled characters will lead to clades with different compositions in the dichotomous 'phylogenetic' trees.

An independent existence of *Bertia* might be warranted in part by the presence of a peculiar kind of ascospore—they are wormlike or cylindrical and curved geniculate, similar to those found in some species of *Lasiosphaeria* Ces. & De Not., including such examples as *L. hirsuta* (Fr.) Ces. & De Not., *L. hispida*

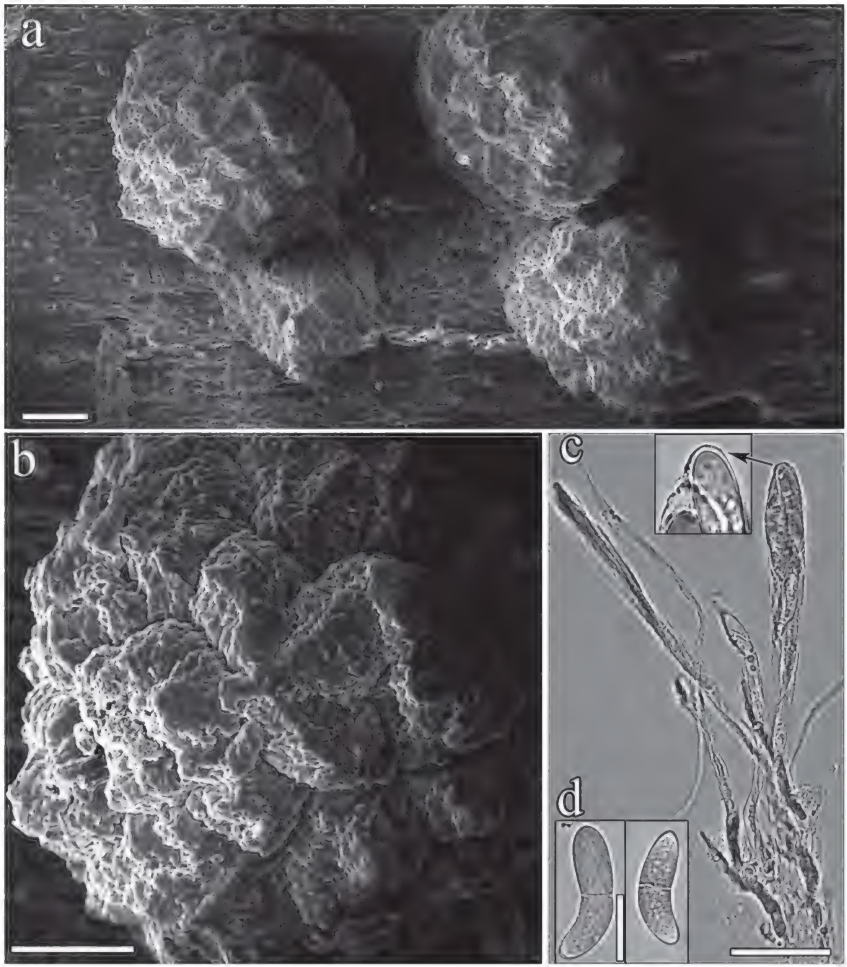

Fig. 2. *Bertia latispora*: (VLA P-2666): a. Ascomata; b. Ascomatal surface; c. Ascus and paraphyses; d. Ascospores. Scale bars: a = 200 μm; b = 100 μm; c = 50 μm; d = 20 μm.

(Tode) Fuckel, and *L. ovina* (Pers.) Ces. & De Not. Ascospore types have been traditionally used to segregate genera in ascomycetous fungi, and we can follow this tradition for the time being.

Acknowledgments

We are grateful to the engineer D.V. Fomin for his assistance in providing the micrographs produced at the Far Eastern Center of Electron Microscopy of the A.V. Zhirmunsky Institute of Marine Biology (Vladivostok). We also thank Prof. Makoto Kakishima (Jilin Agricultural University, China) and Dr. Adam Rollins (Lincoln

Memorial University, USA) for serving as presubmission reviewers and for providing helpful comments and suggestions.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.207

Volume 130, pp. 207–213 *January–March 2015*

Distribution of *Alternaria* species among sections. 1. Section *Porri*

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Abstract — The evaluation of all available descriptions of *Alternaria* species allowed the assignment of another 71 species to *A.* sect. *Porri*, in addition to the 46 species previously included on the basis of phylogenetic analysis. We assess that this section encompasses 117 species, which are listed. An emended and somewhat expanded description of *A.* sect. *Porri* is presented.

Key words — *Nimbya*, *N. rhapontici*

Introduction

The genus *Alternaria* Nees is a large taxonomic group that comprises approximately 280 species (Simmons 2007). A series of large-scale works has attempted to resolve the phylogeny of *Alternaria* and other alternarioid hyphomycetes by employing more than ten different genomic loci (Pryor & Bigelow 2003; Hong et al. 2005; Runa et al. 2009; Lawrence et al. 2012, 2013, 2014; Woudenberg et al. 2013). Several well-supported phylogenetic lineages have been revealed within the alternarioid hyphomycetes that have led to several taxonomic novelties. The genus *Alternaria* was divided into eight taxonomic sections by Lawrence et al. (2013). According to an alternative concept (Woudenberg et al. 2013) all alternarioid hyphomycetes were collapsed into one genus, *Alternaria*, with 24 sections, 11 of which referred to *Alternaria* sensu stricto.

The monophyletic status of the so-called large-spored species was supported by all analyses. Both research groups (Lawrence et al. 2013; Woudenberg et al. 2013) agreed that the group of large-spored species should be treated as a well-defined section of the genus, *Alternaria* sect. *Porri*, based on phylogenetic as well as morphological data. Forty-six species were included in the section

by Lawrence et al. (2013). However, the size of the section was estimated to comprise c. 120 (Gannibal 2012) or c. 80 (Woudenberg et al. 2013) species. *Alternaria porri* was chosen as type species. Only one large-spored species, *A. helianthiinficiens* E.G. Simmons et al., was placed apart from section *Porri* (Woudenberg et al. 2013).

The main discriminating morphological features of many *Alternaria* sect. *Porri* species are larger conidia and the presence of a long filiform apical beak (Lawrence et al. 2013, Woudenberg et al. 2013). Sexual morphs have not yet been discovered. Probably all species in this section are parasites of plants. Species of *A.* sect. *Porri* have a rather strong substrate specialization and usually infect either a single plant species or only few species within a single family.

It is unlikely that all 280 *Alternaria* species will be molecularly characterized in the foreseeable future. Moreover, for several species, living isolates are not known and herbarium material is mostly too old and scanty for successful DNA extractions. On the other hand, the morphological diversity of *Alternaria* is well studied and available in a monograph published by Simmons (2007). Classical comparative morphology can be utilized to establish the affiliation of all *Alternaria* species to a specific section.

The aim of this work was to detect among phylogenetically unexamined *Alternaria* species, as far as possible, all species that fit the current morphological circumscription of *A.* sect. *Porri* and to emend the morphological concept of this section to reflect its extended membership.

Materials & methods

Morphology of almost all *Alternaria* species with legitimate names was analyzed with regard to conformity with criteria of *Alternaria* sect. *Porri*. The morphological assessment was based predominantly on descriptions made by Simmons (2007). Species described after 2007 were assessed using original diagnoses and illustrations. Descriptions of eight species included in *A.* sect. *Porri* were found in Sun & Zhang 2007 (*A. lactucicola* and *A. rhaponticicola*); Labuda et al. 2008 (*A. jesenskae*); Zhang & Zhang 2008 (*A. amphicarpaeae*, *A. sojae*, and *A. tribuli*); and Gannibal 2010 (*A. simmonsii* and *A. silybi*). Two conceptions of *A.* sect. *Porri* were used for comparison, those of Lawrence et al. (2013) and Woudenberg et al. (2013).

Results & discussion

The evaluation of all available descriptions of *Alternaria* species allowed for the addition of 71 species to *A.* sect. *Porri*. The size of this section is assessed to comprise 117 species. *Alternaria* species of *A.* sect. *Porri* are listed in Table 1.

Eight species for which living specimens are not available—*A. bannaensis* W.Q. Chen & T.Y. Zhang, *A. basellae* T.Y. Zhang, *A. calystegiae* Nelen, *A. ellisii* Pandotra & Ganguly, *A. iridicola* (Ellis & Everh.) J.A. Elliott, *A. nicotiana* J.L. Cheng, *A. nyctanthis* K.B. Deshp. & Rajd., and *A. trachelospermicola* (T.Y. Zhang

Table 1. List of *Alternaria* spp. assigned to *A.* sect. *Porri*.

***A. acalyphae* (Nelen) E.G. Simmons [a]**
***A. acalyphicola* E.G. Simmons** (Lawrence et al. 2013)
A. africana E.G. Simmons [b]
***A. agerati* Sawada ex E.G. Simmons** (Lawrence et al. 2013)
***A. agripestis* E.G. Simmons & K. Mort.** (Lawrence et al. 2013)
A. allii Nolla
A. amphicarpaeae Meng Zhang & T.Y. Zhang
***A. anagallidis* A. Raabe** (Lawrence et al. 2013)
A. anodae E.G. Simmons
***A. aragakii* E.G. Simmons** (Lawrence et al. 2013)
***A. argyroxiphii* E.G. Simmons & Aragaki** (Lawrence et al. 2013)
A. ascaloniae E.G. Simmons & C.F. Hill
A. azadirachtae E.G. Simmons & Alcorn
***A. bataticola* Ikata ex W. Yamam.** (de Hoog & Horre 2002; Lawrence et al. 2013)
A. beringelae E.G. Simmons
A. beticola E.G. Simmons & C.F. Hill
***A. blumeae* E.G. Simmons & Sontirat** (Hong et al. 2005; Lawrence et al. 2013)
A. bonducellae R. Dubey et al. [b]
A. brasiliensis F.M. Queiroz et al.
***A. calendulae* Ondřej** (Lawrence et al. 2013)
***A. capsici* E.G. Simmons** (Hong et al. 2005; Lawrence et al. 2013)
A. caricae T.Y. Zhang et al. [b]
***A. carthami* S. Chowdhury** (Lawrence et al. 2013)
***A. cassiae* Jurair & A. Khan** (Lawrence et al. 2013)
A. catharanthi (T.Y. Zhang & X.F. Lin) E.G. Simmons [b]
A. centaureae E.G. Simmons
A. cepulicola V.G. Rao [b]
A. ciceris E.G. Simmons
***A. cichorii* Nattrass** (Lawrence et al. 2013)
***A. cirsinoxia* E.G. Simmons & K. Mort.** (Lawrence et al. 2013)
***A. crassa* (Sacc.) Rands** (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013)
***A. cretica* E.G. Simmons & Vakal.** (Lawrence et al. 2013)
A. cucumericola E.G. Simmons & C.F. Hill
***A. cucumerina* (Ellis & Everh.) J.A. Elliott** (Hong et al. 2005; Lawrence et al. 2013)
A. cyamopsidis Rangaswami & A.V. Rao
***A. cyphomandrae* E.G. Simmons** (Lawrence et al. 2013)
***A. danida* E.G. Simmons** (Lawrence et al. 2013)
A. daturicola T.Y. Zhang et al. [b]
***A. dauci* (J.G. Kühn) J.W. Groves & Skolko** (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
***A. dichondrae* Gambogi et al.** (Lawrence et al. 2013)
A. echinaceae E.G. Simmons & C.F. Hill
A. eichhorniae Nag Raj & Ponnappa
A. enydrae S.A. Khan & M. Kamal [b]
A. euphorbiae (Barthol.) Aragaki & J.Y. Uchida [b]
***A. euphorbiicola* E.G. Simmons & Engelhard** (Hong et al. 2005; Lawrence et al. 2013)
A. flagelloidea (G.F. Atk.) Luttr. [b]
A. gaurae E.G. Simmons & C.F. Hill

A. glyceriae E.G. Simmons & C.F. Hill
***A. grandis* E.G. Simmons** (Lawrence et al. 2013)
A. guangxiensis W.Q. Chen & T.Y. Zhang [b]
***A. hawaiiensis* E.G. Simmons** (Lawrence et al. 2013)
A. heliophytonis E.G. Simmons
A. herbiculinae E.G. Simmons
A. hibiscinficiens E.G. Simmons & C.F. Hill
A. hordeiseminis E.G. Simmons & G.F. Laundon
A. hortensiae Unamuno ex E.G. Simmons [b]
A. iranica E.G. Simmons & Ghosta
A. jesenskae Labuda et al.
A. lactucicola X. Sun & T.Y. Zhang
A. lallemantiae (Khokhr.) E.G. Simmons [c]
A. latispora T.Y. Zhang & Meng Zhang [b]
***A. limicola* E.G. Simmons & M.E. Palm** (Lawrence et al. 2013)
A. linariae (Neerg.) E.G. Simmons
***A. linicola* J.W. Groves & Skolko** (de Hoog & Horre 2002; Lawrence et al. 2013)
A. longirostrata T.Y. Zhang & Meng Zhang [b]
A. loofahae E.G. Simmons & Aragaki
A. lunariae (Oudem. & C.J.J. Hall) E.G. Simmons [b]
***A. macrospora* Zimm.** (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
A. montanica E.G. Simmons & Robeson
***A. multirostrata* E.G. Simmons & C.R. Jacks.** (Lawrence et al. 2013)
A. nattrassii E.G. Simmons [b]
***A. nitrimali* E.G. Simmons & M.E. Palm** (Lawrence et al. 2013)
A. novae-guineensis E.G. Simmons & C.F. Hill
A. obtecta E.G. Simmons
***A. passiflorae* J.H. Simmonds** (Lawrence et al. 2013)
A. petasitis M. Kubota et al. [b]
A. pipionipisi E.G. Simmons
***A. poonensis* Ragunath** (Lawrence et al. 2013)
***A. porri* (Ellis) Cif.** (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
***A. protenta* E.G. Simmons** (Lawrence et al. 2013)
***A. pseudorostrata* E.G. Simmons** (Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
A. pulcherrimae T.Y. Zhang & J.C. David
A. ranunculi E.G. Simmons
A. readeri (G. Winter) Neerg. [b]
A. rhaponticicola X. Sun & T.Y. Zhang
***A. ricini* (Yoshii) Hansf.** (Lawrence et al. 2013)
A. rosa-sinensis M.X. Gao & T.Y. Zhang [b]
A. rosifolii E.G. Simmons & C.F. Hill
***A. rostellata* E.G. Simmons** (Lawrence et al. 2013)
A. saposhnikoviae J.Z. Zhang & T.Y. Zhang [b]
A. sauropodis E.G. Simmons
***A. scorzonerae* (Aderh.) Loer.** (Lawrence et al. 2013)
***A. sesami* (E. Kawam.) Mohanty & Behera** (de Hoog & Horre 2002; Lawrence et al. 2013)

A. sidae E.G. Simmons
A. silybi Gannibal
A. simmonsii Gannibal
A. sojae Meng Zhang & T.Y. Zhang
***A. solani* Sorauer** (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
A. solani-nigri R. Dubey et al. (Lawrence et al. 2013)
A. spinaciae Allesch. & F. Noack [b]
A. stachytarpheticola E.G. Simmons [b]
***A. steviae* Ishiba et al.** (Lawrence et al. 2013)
***A. subcylindrica* E.G. Simmons & R.G. Roberts** (Lawrence et al. 2013)
A. subtropica E.G. Simmons [b]
A. sudanensis E.G. Simmons [b]
A. tabasco E.G. Simmons & R.G. Roberts
***A. tagetica* S.K. Shome & Mustafee** (Lawrence et al. 2013; Woudenberg et al. 2013)
A. thunbergiae E.G. Simmons & Alcorn
A. tillandsiae E.G. Simmons & C.F. Hill
A. tomato (Cooke) L.R. Jones [d]
***A. tomatophila* E.G. Simmons** (Lawrence et al. 2013)
A. tribuli Meng Zhang & T.Y. Zhang
***A. tropica* E.G. Simmons** (Lawrence et al. 2013)
A. vanuatuensis E.G. Simmons & C.F. Hill
A. venezuelensis E.G. Simmons & Rumbos
A. viciae-fabae E.G. Simmons & G.F. Laundon
A. zinniae M.B. Ellis (Lawrence et al. 2013)

Bold font names = species with phylogenetic data (in the cited references).
Unannotated names = species with reliable living cultures and herbarium specimens.
Names with square bracketed annotations = species with herbarium specimens as the only available reliable material:
[a] = representative isolates have not been chosen;.
[b] = no known living isolates;
[c] = isolates are non-sporulating;
[d] = isolates are not correctly identified.

et al.) E.G. Simmons—were found to have intermediate characteristics that do not allow unambiguous placement in *A.* sect. *Porri* or any other section. Dubious morphological traits and the absence of isolates for detailed morphological or molecular studies make it impossible to assess their phylogenetic positions within the genus.

An isolate of *A. tomato* (CBS 114.35, deposited in 1935) clustered with strong support in the autonymous clade, *Alternaria* sect. *Alternaria* (Lawrence et al. 2013). However, the credible species description in Simmons (2007) suggests that this species resides in *A.* sect. *Porri*. According to Simmons (2007), no correctly identified *A. tomato* isolates are known. Hence, we suggest that *A. tomato* sensu Simmons belongs in *A.* sect. *Porri*.

Phylogenetic data revealed the polyphyletic nature of the genus *Nimbya* E.G. Simmons (Lawrence et al. 2012). Only four studied species from *Cyperaceae*

and *Juncaceae* clustered together as a separate clade ("true" *Nimbya*) when three species from *Amaranthaceae* were placed in *Alternaria* sect. *Alternantherae* (Lawrence et al. 2012, 2013). There are five *Nimbya* species found on plants other than *Cyperaceae*, *Juncaceae*, and *Amaranthaceae*, which likely belong to other clades. They have relatively large conidia and some of them could be related to the group of large-spored *Alternaria* species. At least *N. rhapontici* (Nelen) E.G. Simmons is morphologically very similar to *Alternaria* sect. *Porri,* but additional examinations are necessary to clarify the phylogeny and taxonomy of this species.

Here we present an emended and somewhat expanded description of the section:

Alternaria* sect. *Porri D.P. Lawr., Gannibal, Peever & B.M. Pryor, Mycologia 105: 541. 2013; emend. Gannibal.

On V-8 primary conidiophores short, moderately long (up to 150–250 µm) or rarely very long (up to 700 µm or probably even longer [*A. pseudorostrata*, *A. nitrimali*]), simple or branched, with one or few (up to 9 on branched conidiophore [*A. solani*]) conidiogenous loci. Conidia solitary, in short or rarely moderately long chains (up to 7 units on PCA [*A. pseudorostrata*, *A. euphorbiicola*]), simple or with solitary lateral conidia, body of mature conidia moderately large (40–110 × 11–25(–30) µm), broadly ovoid, obclavate, ellipsoid, subcylindrical, or obovoid, disto- and euseptate, slightly constricted at septa, number of transepta in the conidial body varying from 7–9 [*A. cirsinoxia*] to 12–19 [*A. crassa*, *A. grandis*], with a single septum or 2–3 longitudinal septa in one or almost all transverse divisions; conidia of some species almost always without any longitudinal septa [*A. agripestis*, *A. subcylindrica*], pale yellowish tan to dark olivaceous brown, smooth or punctulate, at least some mature conidia with terminal beaks, solitary or in groups (up to 5) appearing as several beaks or a single beak with several branches, beaks aseptate or septate, 50–300(–460) µm long and (4–)5–6(–8) µm wide near the base, apex tapering to 1.5–3 µm or giving rise in secondary conidiophores. Conidia may form lateral or apical secondary conidiophores without filiform portion, sometimes with one or only few lateral secondary conidiophores arising from the body or beak.

In comparison with V-8, on PCA conidia are somewhat larger and have beaks with larger average numbers of branches (Simmons 2007; Gannibal et al. 2014).

Acknowledgments

It is my pleasure to acknowledge the attention of Dr. Uwe Braun and Dr. Daniel Lawrence for their presubmission reviews of this article. This work was supported by Russian Science Foundation (project #14-26-00067).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.215

Volume 130, pp. 215–221 *January–March 2015*

Morchella galilaea, an autumn species from Turkey

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Abstract — *Morchella* specimens collected during the autumn seasons of 2009–12 from Adana Province, Turkey, have been identified by DNA sequencing as representatives of *Morchella galilaea*. The macro- and micromorphology, phylogeny, ecology, and fruiting phenology of the Turkish material are evaluated.

Key words — mist units, morels, taxonomy, volcanic media

Introduction

Morchella species (true morels) are some of the most valuable and important fungi consumed worldwide due to their unique aroma and flavor. Their commercial value has been increasing over the years as a result of growing consumer demand. Countries with diverse morel species include China, USA, Pakistan, India, and Turkey, which export morels to European countries (Pilz et al. 2007). In Turkey, morels generally grow in pine forests (*Pinus brutia*, *P. nigra*) from late February to June (Taşkın & Büyükalaca 2012). According to a review by Pilz et al. (2007), researchers have reported *Morchella* associated with elm, ash, aspen, tulip poplar, apple, and cottonwood trees. Although morel fruiting season occurs generally during the spring season, Sturgis (1905) and Masaphy et al. (2009) have reported morels fruiting in different seasons (Pilz et al. 2007). Morels may be found throughout Turkey, especially in Mediterranean and Aegean regions. If they can be collected in relatively large volumes, morels offer the potential of providing a significant income for people living near the forests and could be regarded as a significant export product for Turkey.

In addition to the existence of an endemic Turkish morel species, *M. anatolica* Işiloğlu et al. (Işiloğlu et al. 2010), Taşkın et al. (2010, 2012)

identified 20 phylogenetically distinct *Morchella* species in Turkey. Nineteen of these species were collected from forests in spring, but one (given the phylogenetic code *Mes*-16) was observed in autumn in the mist units of the Horticultural Research and Application Area of Çukurova University, Adana, Turkey (Taşkın et al. 2012). Based on studies by Taşkın et al. (2012), O'Donnell et al. (2011), Du et al. (2012a,b) we named this population as a new species but later realized that it represented *Morchella galilaea*, described from Israel by Clowez (2012). However, as detailed morphological, microscopical, and ecological features were not provided in the original description, we have evaluated the morphology, phylogenetic position, ecology, and fruiting phenology of *M. galilaea* collected from Turkey. Our results should be beneficial to future studies concerned with culture and conservation of *Morchella* spp.

Materials & methods

Specimens were collected from Adana province in Turkey (Horticultural Research and Application Area of Çukurova University) during autumn and early winter (October–December) between 2009 and 2012 (Fig. 1). Two (in 2009), two (in 2010), and 11 (in 2012) *Morchella* specimens were collected from mist units used for rooting agricultural plant cuttings.

Molecular analyses were performed at the United States Department of Agriculture (USDA-NCAUR-Peoria-IL) in USA (Taşkın et al. 2012). These samples were analyzed through partial RNA polymerase I (RPB1), RNA polymerase II (RPB2), translation elongation factor (EF1-α), 28S (LSU) rRNA gene and internal transcribed spacer (ITS) rRNA gene sequences. Macro- and microscopical characters were studied at the Department of Biology, Selçuk University (Konya, Turkey). The specimens were mounted in Melzer's reagent and examined microscopically using a Leica DM 750 microscope. Volcanic tuff was analyzed at Alata Horticultural Research Institute, and pH (Jackson 1962), salinity (Soil Survey Division Staff 1993), total lime ($CaCO_3$; Çağlar 1949), organic matter (Walkley & Black 1934), phosphorus (P; Olsen & Dean 1965), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) were analyzed using an atomic absorption spectrophotometer (Varian SpectrAA Model 220FS, Germany).

Taxonomy

Morchella galilaea Masaphy & Clowez, Bull. Soc. Mycol. Fr. 126 (3-4): 238. 2012 ["2010"]. Figs 1–2

Ascomata 53–78 mm tall. Hymenophore 36–45 mm tall, 12.5–17.5 mm wide, conic to subcylindric, pitted and ridged, ridges have two types, these are narrow polygonal ridges and long elliptical ridges. Sterile Ribs longitudinally arranged, 5.2–9.8 mm wide, 14.5–24.74 mm tall, thin, elastic, simple, or forked and anastomosing to form elongated hymenial pits, white, bluntly rounded when young, but often flattened in age, ridges glabrous.

Fig. 1 – *Morchella galilaea* specimens collected from mist units on the Çukurova University campus (Adana, Turkey).

Hymenial Pits light gray, whitish gray, blackish gray or silvery gray, when young; becoming, greyish-light brown, light olive brown or yellow brown with maturity. Ascocarp margin connecting directly into the stalk without a sharp bend, free. Stipe 17–33 mm high, 5–8.2 mm wide, cylindrical and thickening slightly towards the base; surface when young completely glabrous and white, in maturity light yellowish white. Context whitish, 0.5–1 mm thick in the hollow hymenophore; sterile inner surface whitish, glabrous. Ascospores elliptical, smooth (surface distinctly rugulate to tectate under the scanning electron microscope), with homogeneous contents, (12.5–)13.5–18(–19.5) × (7.5–)8–9.5(–10) µm [average Q =1.83]. Asci 8-spored, 165–220 × 15–22 µm, cylindric, hyaline. Paraphyses cylindric to subclavate, apices rounded to subacute, with 2–3-septate, hyaline or with brown to brownish homogeneous contents in KOH (2%), 100–130 × 6–11 µm. Residual Paraphyses on sterile ridges similar to paraphyses, hyaline or with bundled brownish contents.

Specimens Collected: Turkey. Adana: Çukurova University Campus, 37°01′55″N 35°22′12″E, alt. 39 m, solitary or scattered on volcanic tuff in misted cutting propagation beds, 17 Nov 2009, leg. H. Taşkın, Taskin 117 (ANK); 28 Nov 2012, leg. H.H. Doğan, Doğan 10022 (Kon Fungarium).

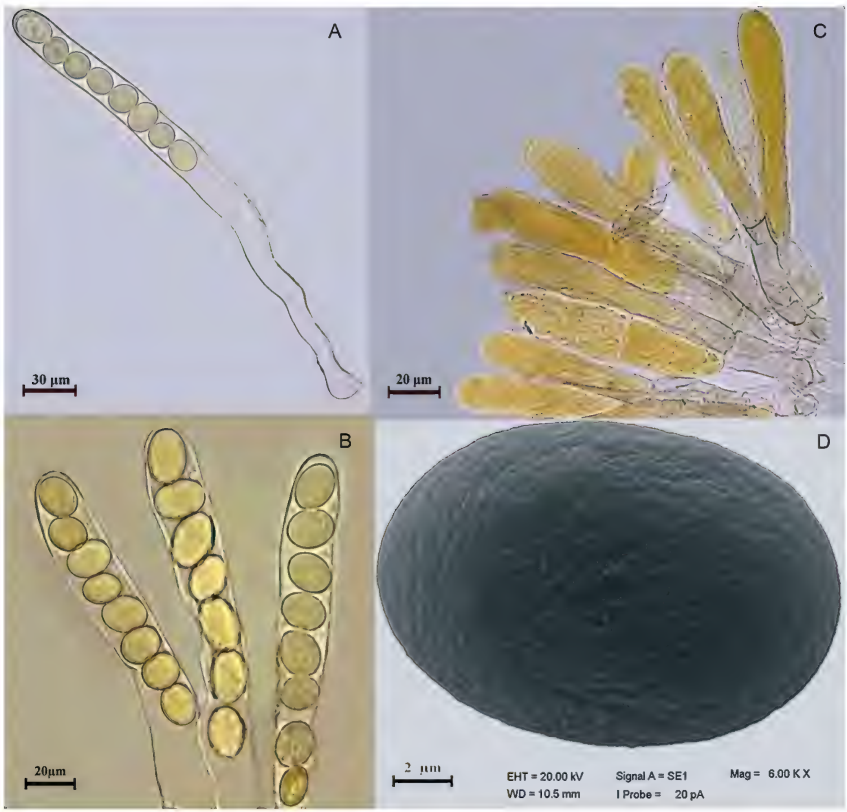

Fig. 2 – *Morchella galilaea* (Turkish specimen, Taskin 117).
A, B: asci with ascospores. C: paraphyses. D: ascospore (SEM).

Discussion

The morphological features of *M. galilaea* were cited by Clowez (2012) as: ascomata 3–5 cm height, ovoid, light yellow; primary alveolus large, few; edges of ribs same color and rusty; stipe cylindrical rather slender, attenuated at the top and wider at the base.

When we compared *M. galilaea* samples collected from Israel and Turkey morphologically, we found certain differences between the samples, which we summarize as follows: (a) ascomata of the Israel specimens were shorter (3–5 cm); (b) the stipes of the Turkish specimens were more or less cylindrical except for the slightly thicker base while in the Israeli collection the stipes had a narrower apex and wider base; (c) the Israeli specimen was found under *Fraxinus syriaca,* while our Çukurova University specimens were collected from a no tree zone.

Molecular characterization

Morchella galilaea [as *Mes*-16] has been molecularly assessed through GCPSR analyses by Taşkın et al. (2012) and also molecularly analyzed by O'Donnell et al. (2011), Du et al. (2012 a, b), Masaphy et al. (2010), Clowez (2012), and Richard et al. (2015). These studies indicate that *M. galilaea* occurs in China (Du et al. 2012a,b), Java, Hawaii, Israel (Masaphy et al. 2010, Clowez 2012), New Zealand, India (Kanwal et al. 2011), and three African countries as well as in Turkey. The Turkish and Israel sequences of *Morchella galilaea* are deposited in GenBank-ITS1-5.8S-ITS2 as JQ723082 and GU589858, respectively (Masaphy et al. 2010; Clowez 2012; Du et al. 2012 a, b).

Ecological assessment

The results of the volcanic tuff analyses are provided in Table 1. Relatively high levels of copper, manganese, zinc, iron, magnesium and calcium were detected in the volcanic tuff medium, which was identified as having a loam soil structure, normal $CaCO_3$, and alkali salinity profile. There was a relatively low potassium level. Singh et al. (2004), who determined that pH values vary from 6.5 to 7 in morel natural areas in India, detected high levels of calcium, carbon, nitrogen, sodium, and lead and low levels of phosphates, chloride, and potassium; soil type varied from sandy to loam.

Sturgis (1905) observed morels fruiting in September in an aspen-spruce forest burned the previous summer in southwestern British Columbia (Pilz et al. 2007). Masaphy et al. (2009) reported that a *Morchella* species emerged from late autumn to late spring in northern Israel under *Rhamnus alaternus*,

Table 1. Properties of the volcanic tuff in which *Morchella galilaea* was found fruiting.

Property	Results
Texture (100 g/ml)	46
$CaCO_3$ (%)	19.6
Salinity (mmhos/cm)	0.06
Organic matter (%)	2.6
pH	7.6
Available potassium (ppm)	167.5
Available phosphorus (ppm)	19.2
Calcium (ppm)	2300
Available magnesium (ppm)	759
Iron (ppm)	6.6
Zinc (ppm)	2.8
Manganese (ppm)	4.8
Copper (ppm)	1.6

Platanus orientalis, *Populus euphratica*, *Laurus nobilis*, and *Fraxinus syriaca*. However, they could not find a mycorrhizal relationship between trees and morels. *Morchella* is known to be saprobic. However, according to a review study conducted by Tedersoo et al. (2010), *Morchella* species may associate weakly with plant roots and thus are likely to be facultative biotrophs. Nonetheless, some researchers believe that certain *Morchella* species may be able to form mycorrhizal relationships with certain trees. Dahlstrom et al. (2000) reported a mycorrhizal relationship between *Morchella* and species of *Pinaceae,* and Buscot & Kottke (1990) detected a mycorrhizal association between *Morchella* and *Picea abies*. Our morel specimens grew in a mist unit. While this unit was used for rooting olive cuttings during 2009–2010, no cuttings were placed into the units in 2012, which suggests that our specimens are probably not mycorrhizal. Our hypothesis is that the mycelium of the species was carried to the mist units by means of the volcanic tuff, where it developed over the years. After mycelial development, a sprinkle irrigation system and hormone application used for rooting might also have stimulated *Morchella* fruiting. The same species has been found under Populus and Quercus by Du et al. (2012b) and under *Fraxinus syriaca* by Masaphy et al. (2010). Although morels typically fruit from late February to early June, our *Morchella* specimens were collected between October and December. In 2012, the air temperature at Çukurova University-Adana averaged 29°C in October, 23°C in November, and 16°C in December.

Acknowledgements

The authors would like to express their sincere gratitude to Dr. Kerry O'Donnell (USDA-NCAUR-Peoria, IL, USA) for allowing us to perform molecular analyses in his laboratory. A special thanks goes to Dr. Davut Keleş (Manager of Alata Horticultural Research Institute), for the volcanic tuff analysis, and to Berken Çimen, for organization of the figures. We are also indebted to Prof. Dr. Mitko Karadelev, Prof. Dr. Aysun Pekşen, Prof. Dr. Daniel Royse for their critical reviews of the manuscript.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.223

Volume 130, pp. 223–225 *January–March 2015*

Hyphoderma hallenbergii, a new corticioid species from India

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Abstract — *Hyphoderma hallenbergii* sp. nov. from Himachal Pradesh, India is described.

Key words — Baghi, *Basidiomycota*, *Agaricomycetes*, *Meruliaceae*

During August 2012 fungal forays conducted of the Baghi area of district Shimla, Himachal Pradesh, India, Maninder and Avneet collected a fungus on decaying wood of *Cedrus deodara* in a mixed forest. Comparison of macroscopic and microscopic characters (Eriksson & Ryvarden 1975, Dhingra 1989, Larsson 1998, Bernicchia & Gorjón 2010, Singh et al. 2010, Priyanka & Dhingra 2012, Mycobank 2014) suggested that it belonged in the genus *Hyphoderma* (*Meruliaceae*, *Agaricomycetes*, *Basidiomycota*). The fungus, which is similar to *H. nemorale* K.H. Larss. and *H. incrustatum* K.H. Larss. in having two types of cystidia but which differs in basidiospore size, is described here as a new species.

Hyphoderma hallenbergii Man. Kaur, Avneet P. Singh & Dhingra, **sp. nov.** Plate 1

MycoBank MB 809801

Differs from *Hyphoderma nemorale* and *H. incrustatum* by its smaller and ellipsoid basidiospores.

Type: India, Himachal Pradesh: Shimla, Narkanda, on Baghi road, on the bark of decaying wood of *Cedrus deodara* (Roxb. ex D. Don) G. Don, 19 August 2012, Maninder Kaur & Avneet 6962 (PUN, **holotype**).

Etymology: In the honor of Dr. Nils Hallenberg, Professor Emeritus, University of Gothenburg, Sweden.

Basidiocarp resupinate, adnate, effused, ≤100 µm thick in section; hymenial surface smooth to somewhat grandinioid, chalky white; margins thinning

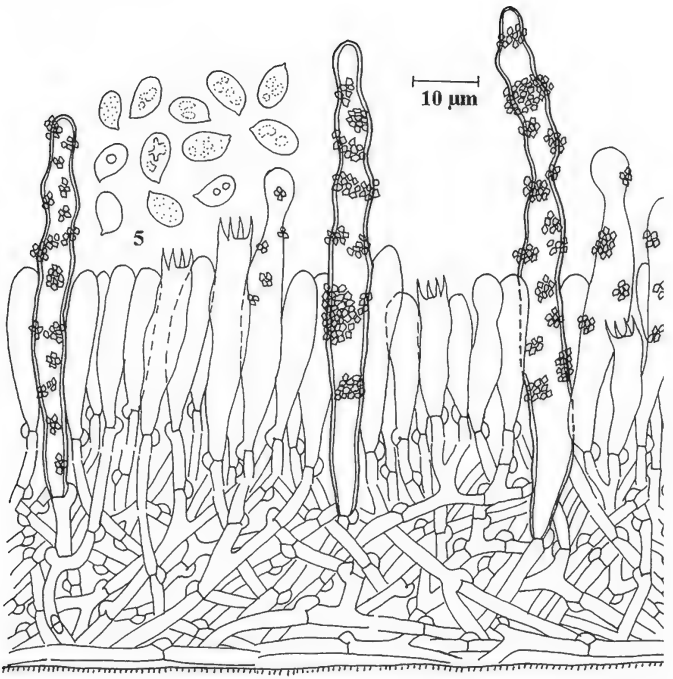

Plate 1. *Hyphoderma hallenbergii* (holotype). 1. Basidiocarp showing hymenial surface. 2. Capitate hymenial cystidia. 3. Basidium. 4,5. Basidiospores. 6. Vertical section through basidiocarp.

out or indeterminate, concolorous or paler. Hyphal system monomitic. Generative hyphae ≤2.5 µm wide, branched, septate, clamped; subiculum poorly developed, formed of few hyphae running more or less parallel to the substrate; subhymenial hyphae well developed, vertical, denser. Cystidia of two types: [1] Capitate cystidia 25–44 × 5–6 µm, subfusiform, originating from the hymenium, thin-walled, with basal clamp, sparsely encrusted; [2] Cystidia which are cylindrical to flexuous with constrictions, sometimes almost moniliform 60–80 × 5–7 µm, originating from subhymenium, slightly thick-walled, with basal clamp, often encrusted with crystals. Basidia 16–34 × 4–5 µm, clavate to subclavate, constricted to sinuous, 4-sterigmate, with basal clamp; sterigmata ≤3.5 µm long. Basidiospores 6.3–7.5 × 3.5–4.5 µm, ellipsoid, with oily contents, thin-walled, inamyloid, acyanophilous.

Remarks — *Hyphoderma nemorale* and *H. incrustatum* both differ from *H. hallenbergii* by their larger, cylindrical basidiospores (*H. nemorale*, 10–14 × 4–5 µm; *H. incrustatum*, 11–16 × 4–5.5 µm; Larsson 1998).

Acknowledgements

The authors thank Head (Department of Botany, Punjabi University, Patiala) for providing research facilities, Dr. Nils Hallenberg (Professor Emeritus, Denmark) for expert comments and peer review; and Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.227

Volume 130, pp. 227–231 *January–March 2015*

Xylaria thailandica – a new species from southern Thailand

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Abstract —A new species, *Xylaria thailandica*, was collected from dead wood and fallen branches in southern Thailand. This fungus has upright, gregarious, cylindrical stromata that arise from a tomentose stipe and which is internally dark brown to black at the center but yellow around the perithecia. Its ascospores are inequilateral and with a germ slit that spirals lengthwise.

Key words — ascomycetes, *Xylariaceae,* decaying wood, fallen branches

Introduction

Fungi belonging in the genus *Xylaria* are saprophytic, endophytic, parasitic (Whalley 1985), or symbionts in insect nests (Hawksworth et al. 1995, Pélaez et al. 2008). They are characterized by upright, sessile, or stipitate stromata with cylindrical, clavate, or irregularly shaped fertile parts. Many species have been reported from different substrata throughout the world (Rogers & Samuels 1986, Bi et al. 1993, Rogers et al. 2005, Ju & Hsieh 2007, Ju et al. 2009, Rogers et al. 2008, Carmona et al. 2009, Hladki & Romero 2010, Fan & Lin 2011, Fournier et al. 2011, Rogers & Ju 2012).

Thailand is considered one area containing a high percentage of *Xylaria* species, and several studies have reported on their biodiversity (Carroll 1963, Schumacher 1982, Thienhirun 1997, Ruksawong & Flegal 2001, Chansrikul et al. 2008, Petcharat et al. 2012). As currently recognized, *Xylaria* is represented

in Thailand by 42 species. One recent collection that could not be identified as any known *Xylaria* species is described and illustrated herein as *Xylaria thailandica*.

Materials & methods

The specimens were collected from a natural plant site in a tropical rain forest in southern Thailand. Hand-cut sections of dried material revived in 70% ethyl alcohol were examined microscopically. Ascospore measurements were made from squash mounts in lactophenol and the dimensions were determined from 20 fully mature spores. A drop of Melzer's reagent was added to determine the amyloid iodine reaction. The photographs of stromata were taken using a Canon 550D DLSR digital camera. Cultures were obtained from ascospores using the single spore isolation technique and characterized on Potato Dextrose Agar (PDA) at room temperature (28–30 °C). All the specimens are kept at Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hatyai, Songkhla, Thailand (PSU-PM).

Taxonomy

Xylaria thailandica Srihanant, Petcharat & Lar.N. Vassiljeva, **sp. nov.** Fig. 1

MycoBank MB 807720

Differs from other species of *Xylaria* by its yellow-colored flesh, ascospores with a spiraling germ slit, and asci with an urn-shaped apical ring.

Type—Thailand, Nakorn si thammarat Province, para rubber plantation, on dead wood and fallen branches, 26. X. 2010, Natthapach Srihanant, (**holotype** PSU-PM 1059).

Etymology—Refers to Thailand, where this fungus was collected.

Stromata upright, gregarious, cylindrical, unbranched or (sometimes) branched at the stipe, with short to long acute sterile apices, 3–7 cm total length × 0.1–0.2 cm diam, externally at first brownish, becoming dark brown, then black; internally dark brown to black at core, but yellow around perithecia, texture soft, surface roughened by remnants of peeling layer and short dark brown hairs; hairs not evident in old material. Young stroma filiform, dark brown, but dull green at the top where the fruiting bodies produce conidia (asexual spores). Stipe short to long, filiform, with a broadened discoid base, covered with short brown hairs. Perithecia completely immersed, globose to spherical, 210–310 μm high × 135–250 μm diam, ostioles papillate. Asci 1.5–2 × 1–1.5 μm, apical ring urn-shaped, blue in Melzer's. Ascospores brown, unicellular, inequilateral, with broadly or narrowly rounded ends, 9–14.5 × 3–4.8 μm, with spiraling germ slit spore-length.

Colonies on PDA: Colonies growth 14 days at room temperature (28–30 °C) on PDA 9 cm diam., mycelium cottony, white with black color on the surface of the agar. Stromatic structures not forming on media.

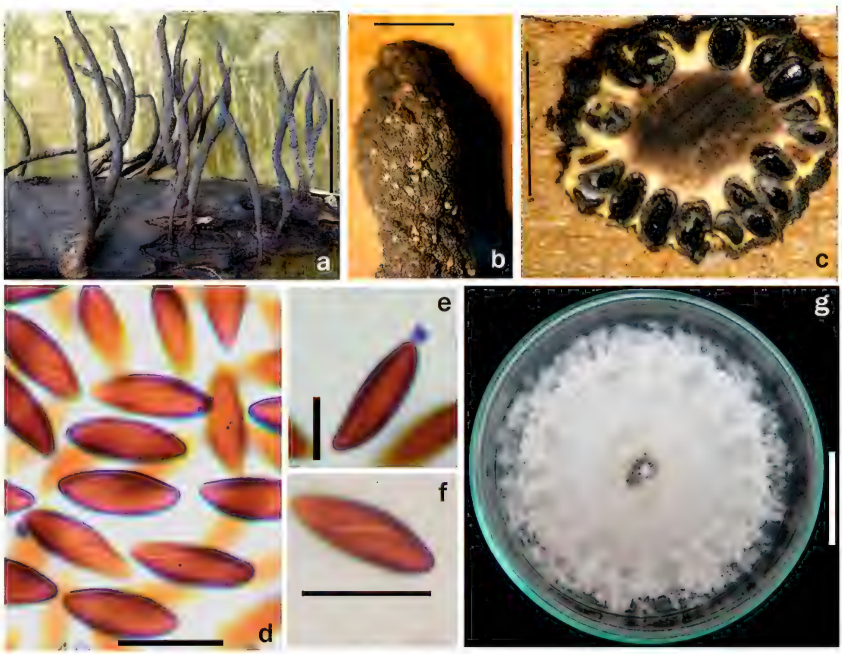

Fig. 1. *Xylaria thailandica* (holotype). A. Mature teleomorphic stroma on dead wood. B. Stromata surface with conspicuous perithecial mounds. C. Transverse section of a stroma with dark color at the core. D. Ascospores in lactophenol. E. Ascospore in Melzer's reagent with an urn-shaped apical ring. F. Ascospore in lactophenol with spiral germ slit. G. Colonies on PDA in a 5 cm petri dish at 10 days. Scale bars: A = 2 cm; B, C = 1 mm; D = 10 µm; E = 5 µm; F = 10 µm; G = 1 cm

Additional specimens examined—**THAILAND**, **Songkhla Province**, Prince of Songkla University, Khao khohong nature trail, on fallen branches 5.XII.2010, Natthapach Srihanant (PSU-PM 1060); **Suratthani Province**, oil palm plantation, on dead wood in 24. I. 2011, Natthapach Srihanant (PSU-PM 1061).

Comments—*Xylaria thailandica* differs from other species on wood (Fournier et al. 2011, Hladki & Romero 2010, Ju et al. 2009, Rogers & Samuels 1986, Roger et al. 2008) by its yellow flesh and ascospores with spiraling germ slits.

This species can be separated from *X. longipes* var. *tropica* F. San Martín & J.D. Rogers (Thienhirun 1997) and *X. longipes* Nitschke (Rogers et al. 2008), which have larger ascospores and asci with a differently shaped apical apparatus.

The stromatal morphology of *X. thailandica* is close to *X. cinerea* J. Fournier & M. Stadler (Fournier et al. 2011), which differs by its larger ascospores with a slightly sinuous germ slit.

Comparisons between *X. thailandica* and other species are shown in Table 1.

Table 1. Comparison of some wood-inhabiting species of *Xylaria* with the new taxon *X. thailandica*.

Species	Spore size (µm)	Germination slit	Apical apparatus
X. thailandica	9–14.5 × 3–4.8	Spiral, full spore-length	Urn-shaped
X. apiculata	16–30 × 5–9	Straight, full spore-length	Minute
X. castorea	8–14 × 4.5–6.5	Straight, probably full length	Minute
X. cinerea	13–17 × 5–6	Straight to slightly sinuous	Tubular to urn-shaped
X. grammica	12–14.5 × 4–6.5	Straight, full spore-length	Rectangular
X. hypoxylon	10–15.5 × 4.5–6.5	Straight to undulate, less than full length	Wedge-shaped
X. karsticola	11–14.5 × 4.2–6	Conspicuous straight, full spore length	Tubular with a slightly flared apex
X. longipes	9–15 × 5–6	Spiral, around spore	Not seen
X. longipes var. *tropica*	14–16.5 × 5–6	Spiral, full spore-length	Quadrate to rectangular
X. multiplex	9.5–11 × 4.5–5	Straight, almost full spore length at the ventral side	Not seen
X. zealandica	22–31 × 7.5–9	Straight to undulate, slightly less than spore length	Urn-shaped

Acknowledgments

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission. The first author would like to acknowledge Department of Pest Management, Prince of Songkla University, providing necessary facilities in this research. We are thankful to Dr. Julia Checa (Universidad de Alcalá, Madrid, Spain) and Dr. Chaninun Pornsuriya (Prince of Songkla University, Songkhla, Thailand) for reviewing the manuscript of our paper and for their valuable suggestions.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.233

Volume 130, pp. 233–236 *January–March 2015*

Rosellinia brunneola sp. nov. and *R. beccariana* new to China

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Abstract — A new species, *Rosellinia brunneola*, is described from Hainan Province, China; and *Rosellinia beccariana* from Yunnan Province is described as new to China.

Key words —*Ascomycota*, pyrenomycetous fungi, *Xylariaceae*, taxonomy

Rosellinia species, which are cosmopolitan in their distribution, are especially rich in tropical and subtropical regions. 142 species have been recognized (Petrini 2013). During mycological expeditions to tropical regions of Hainan and Yunnan Provinces, many specimens of *Rosellinia* have been collected, from which a new species and a new Chinese record are described here.

Rosellinia brunneola Wei Li bis & L. Guo, **sp. nov.** Figs 1–3

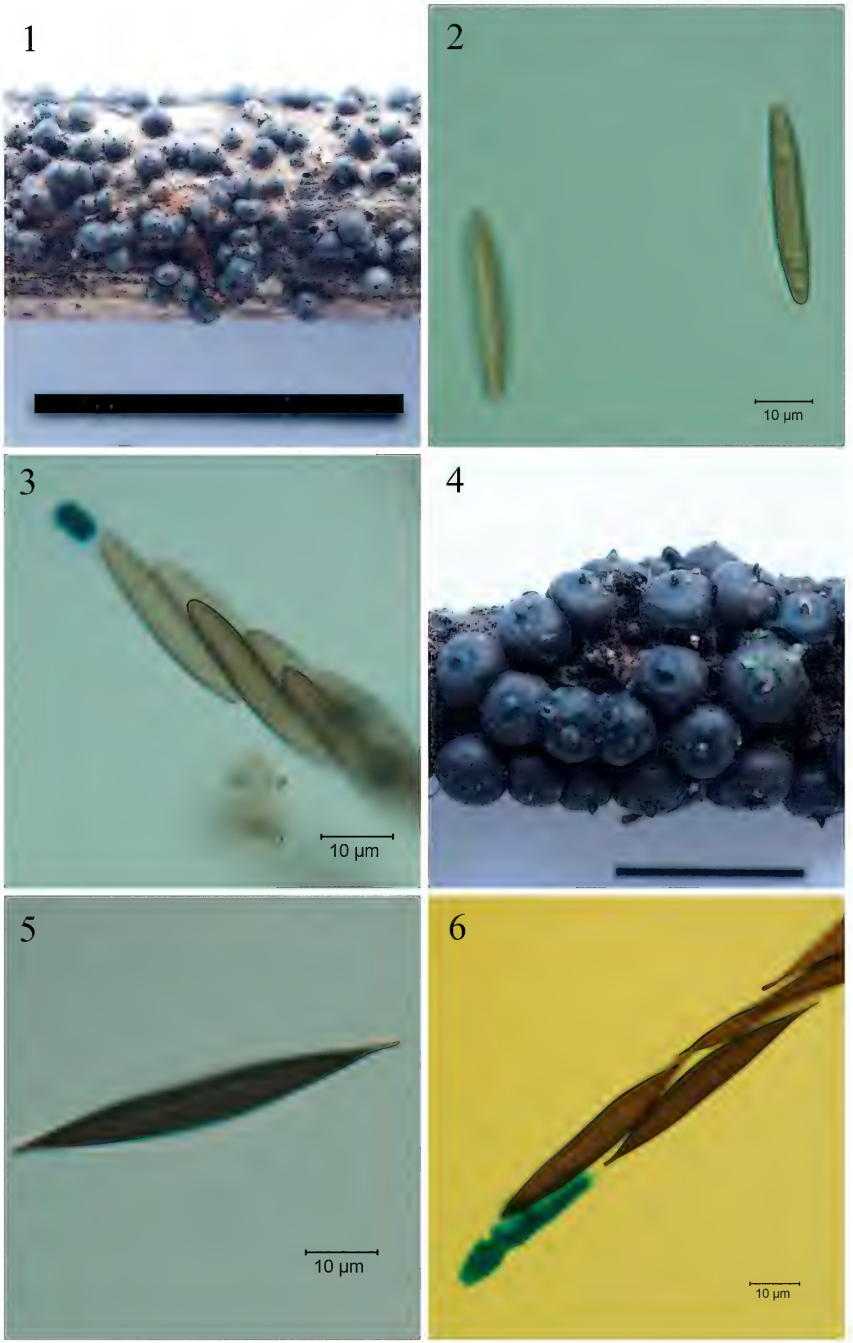
Fungal Name FN 570115

Differs from *Rosellinia mastoidiformis* by its smaller stromata and ascospores with a straight germ slit that is as long as spore length or almost so.

Type: China, Hainan, Lingao, Juliu, alt. 250 m, on dead stems, 4.XII.2010, L. Guo 11546 (HMAS 269893, **holotype**).

Etymology: The epithet refers to the color of ascospores.

Stromata gregarious or solitary, 0.44–0.7 mm high, 0.6–0.8 mm wide, semiglobose or coniform, with top tapering to a point, surface smooth, black. Ostioles papillate. Subiculum not seen. Ectostroma 30–60 μm thick, black. Entostroma white or brownish. Asci eight-spored, with spores arranged in biseriate manner, cylindrical, the spore-bearing part 100–120 × 10–12.5 μm, with apical ring bluing in Melzer's iodine reagent, cylindrical, 6–7 μm high, 3–4 μm broad. Ascospores brownish, asymmetrically ellipsoidal, with rounded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ends, 31–39 × 5–7 µm (33.8 ± 3.2 × 5.9 ± 1.1 µm; 95%CI: 32–35 µm, 5.5–6.5 µm), with straight germ slit, 26–34 µm long.

Comments: *Rosellinia brunneola* is similar to *R. mastoidiformis* Saccas, which differs by having larger stromata (0.5–0.9 mm high, 0.6–1 mm wide) and ascospores with straight and indistinct germ slit that is shorter than spore length (Petrini 2013).

Rosellinia beccariana Ces., Atti Accad. Sci. Fis. Mat. Napoli 5(21): 12. 1872.

Figs 4–6

Stromata gregarious or solitary, subglobose, sometimes with a broad, short stipe, copper brown or black, 2.25–3.7 mm high, 2.9–3.75 mm wide, surface smooth. Ostioles up to 600 µm, pointed. Ectostroma 150–200 µm thick, black. Perithecia subglobose, 1.7–3.15 mm high, 2.6–3.5 mm wide. Asci eight-spored, with spores arranged in biseriate manner, cylindrical, 278–355 µm total length, 10–12.5(–15) µm broad, the spore bearing part 201–248 µm long, with apical ring bluing in Melzer's iodine reagent, arrowhead-shaped, 25–27 µm high, 5–8 µm broad. Ascospores brownish or brown, unicellular, asymmetrically fusiform, with thread-like ends, 48–68 × 6–9 µm, with straight germ slit shorter than spore-length on flat side.

Comments: The ends of the ascospores in the Chinese material lack the slimy sheath, which is absent in old material (Petrini 2013). The large stromata with markedly acute ostioles make this fungus easy to recognize.

Specimen examined: **China**, **Yunnan**, Mengla, Menglun, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, alt. 570 m, on corticated wood, 15.X.2013, W. Li 2903 (HMAS 253034).

Including the two species reported in this paper, 36 *Rosellinia* species have been recorded in China (Teng 1963, Tai 1979, Ju & Rogers 1990, 1999, Yuan & Zhao 1993, Liu et al. 2010, Petrini 2013).

Acknowledgements

The authors would like to express their deep thanks to Dr. L. Vasilyeva (Vladivostok, Russia) and L.E. Petrini (Breganzona, Switzerland) for serving as pre-submission reviewers, to Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review, and to the support by the Ministry of Science and Technology of the People's Republic of China (No. 2013FY110400).

Figs 1–3. ***Rosellinia brunneola*** (HMAS 269893, holotype). 1. Stromata on dead stems (bar = 1 cm); 2. Ascospores; 3. Ascus. Figs 4–6. ***Rosellinia beccariana*** (HMAS 253034). 4. Stromata on wood (bar = 1 cm); 5. Ascospores; 6. Ascus.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.237

Volume 130, pp. 237–239 *January–March 2015*

Phyllachora hainanensis sp. nov. from China

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Abstract — A new species, *Phyllachora hainanensis* on *Phyllanthus emblica* (*Phyllanthaceae*), causing tar spots is reported from Hainan Province, China. *Phyllachora hainanensis* differs from other similar species in the size and shape of ascospores.

Key words — *Sordariomycetes*, taxonomy

A new species of *Phyllachora* (*Sordariomycetes*) on leaves of *Phyllanthus emblica* was collected from Hainan Province, China, in November 2007. Worldwide, only *P. phyllanthophila* and *P. phyllanthophila* var. *egregia* have previously been reported from the host genus *Phyllanthus* (Saccardo & Saccardo 1905: 834, Saccardo & Trotter 1913: 419).

Phyllachora hainanensis Na Liu & G. Huang, **sp. nov.** Plate 1

Fungal Name FN570110

Differs from *Phyllachora phyllanthophila* by its larger ascospores.

Type: China, Hainan Province, Xiawangling, alt. 800 m, on leaves of *Phyllanthus emblica* L. (*Phyllanthaceae*), 13 XI 2007, Shuanghui He, L. Guo & Z.Y. Li 2013 (HMAS 245338, **holotype**).

Etymology: From Hainan Province, where the new species was collected.

Ascomata epigenous, raised, shiny, black, sparse, subglobose, ellipsoidal or irregular, approx. 370 × 150–260 μm diam., immersed in the mesophyll, usually with a single locule, ostiole conspicuous with a conical neck extending through the host epidermis and cuticle, without periphyses. Asci arise from the basal and lateral walls of the ascoma, upper wall to 50 μm thick, composed of epidermal cells occluded by melanised material. Asci 60–100 × 30–70 μm,

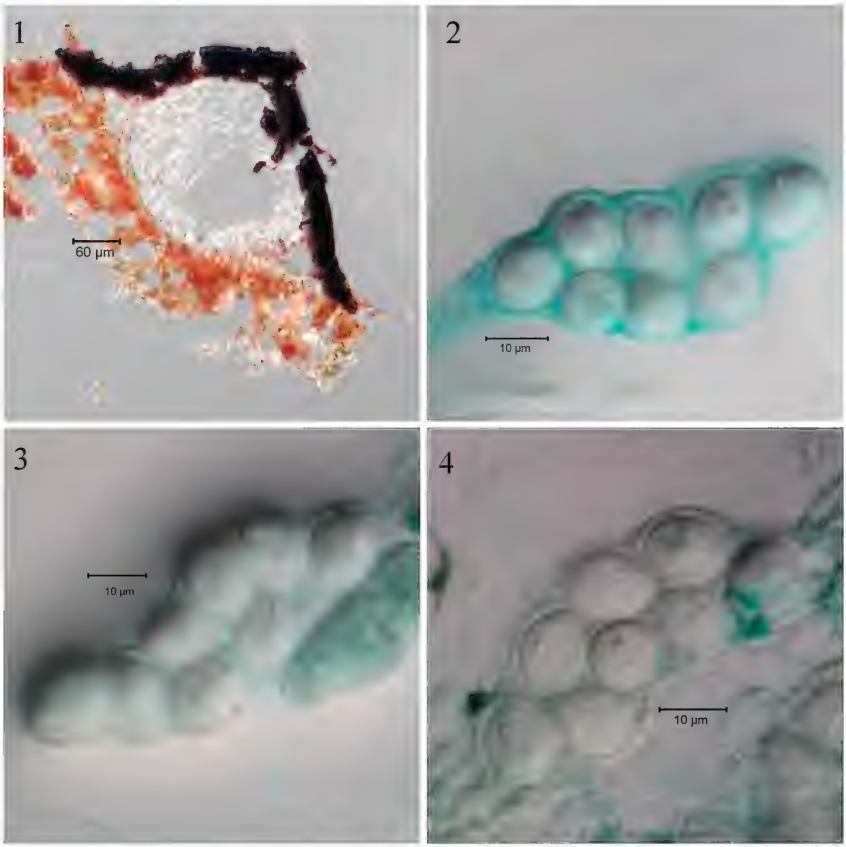

Plate 1. *Phyllachora hainanensis* (HMAS 245338, holotype).
1. Ascomata; 2–4. Asci and ascospores.

8-spored, clavate, obtuse at apex, long pedunculate, thin-walled at maturity, unitunicate. Ascospores biseriate or irregularly biseriate, 11–18 × 9–13 µm, ellipsoidal or subglobose, one-celled, hyaline, thin-walled, smooth, without a gelatinous sheath. Anamorph not seen.

Comments—Subglobose ascospores are rare in *Phyllachora*. Only one other species, *P. yunnanensis* Na Liu & L. Guo, collected on *Lespedeza* (*Fabaceae*) from Yunnan Province of China, has been reported as having subglobose (sometimes ellipsoidal) ascospores. *Phyllachora yunnanensis* differs from *P. hainanensis* in its smaller (7–13.5 × 5–9 µm) ascospores (Liu & Guo 2006).

Phyllachora hainanensis is the first *Phyllachora* species in China discovered on a host species in the *Phyllanthaceae*. Most *Phyllachora* species reported

in China occur on hosts in the *Poaceae, Fabaceae,* and *Cyperaceae* (Tai 1979, Teng 1963, Lan et al. 2012, Cui 2009). The only other reports of *Phyllachora* on *Phyllanthus* are *Phyllachora phyllanthophila* Henn. on *Phyllanthus* sp. from Peru (Hennings 1904: 250) and *P. phyllanthophila* var. *egregia* Rehm on *Phyllanthus* sp. from Brazil (Rehm 1907: 530). These taxa were subsequently transferred to *Catacauma* as *C. phyllanthophilum* (Henn.) Theiss. & Syd. and *C. egregium* (Rehm) Theiss. & Syd. (Theissen & Sydow 1915: 390–391), but *Catacauma* is now synonymised with *Phyllachora* (von Arx & Müller 1954: 212) and *P. phyllanthophila* var. *egregia* is synonymised with *P. phyllanthophila* (Index Fungorum 2015). The publications cited above provide different sets of measurements that can be summarised as 35–60 × 12–20 µm for asci, and 10–14 × 5–9 µm for ascospores; thus *P. phyllanthophila* differs from *P. hainanensis* by its smaller asci and its narrower, ellipsoidal to subovoid ascospores.

Acknowledgments

The authors would like to thank Dr. Roger Shivas (Brisbane, Australia) and Guangyu Sun (Northwest Agriculture and Forestry University) for serving as pre-submission reviewers, Drs. Shuanghui He and Zhenying Li for collecting the specimen, Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review, and Mr. Ziyu Cao (Institute of Botany, Chinese Academy of Sciences) for identifying the host plants. This study was supported by the National Natural Science Foundation of China (No. 31300015) and Natural Science Research Project of Henan Province (No. 132300410350).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.241

Volume 130, pp. 241–246 *January–March 2015*

Two new species of *Zasmidium* from Nepal

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Abstract — *Zasmidium cassines* on living leaves of *Cassine glauca* (*Celastraceae*) and *Z. fabaceicola* on living leaves of *Vigna unguiculata* (*Fabaceae*) are described and illustrated from a forest in the terai region of Nepal. The two new foliar fungi are compared with closely related taxa.

Key words — foliar diseases, hyphomycetes, morphotaxonomy

Introduction

During a 1993–94 survey of anamorphic foliar fungi in the forest of the terai region of Nepal, two undescribed species of *Zasmidium* were discovered. These are characterized by having planate conidial scars, verruculose superficial hyphae, and solitary to rarely catenate and usually rough-walled conidia. Verruculose superficial hyphae and rough-walled conidia are characters shared by both *Stenella* and *Zasmidium*. Molecular analyses (Arzanlou et al. 2007) have shown that the genus *Stenella* is polyphyletic and that its type species, *S. araguata* Syd. (Sydow 1930), clusters within the *Teratosphaeriaceae*. This species is characterized by pileate conidiogenous loci, while other former *Stenella* species are distinguished by having planate *Cercospora*-like conidiogenous loci and belong to the *Mycosphaerellaceae*. Arzanlou et al. (2007) also demonstrated that *Zasmidium cellare* (Pers.) Fr. (Fries 1849), the type species of the genus *Zasmidium*, clusters within the *Mycosphaerellaceae* and agrees both morphologically and phylogenetically with *Stenella*-like hyphomycetes now included in the that family. Hence, the name *Zasmidium* is

available for all former *Stenella* species characterized by planate conidial scars clustering in the *Mycosphaerellaceae*.

Materials & methods

Diseased leaves were collected and dried to make herbarium specimens and deposited in Herbarium Cryptogamiae Indiae Orientalis, Indian Agricultural Research Institute, New Delhi, India (HCIO). Isotypes are retained in the herbarium of the Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India (GPU) for further reference. Microscopic slides of fungal structures were prepared in lactophenol and cotton-blue mixture from infected area of leaf. Observations were made with light microscope using oil immersion (1000×). The figures of fungal structures were drawn using a camera lucida and show all necessary details of morphology and ontogeny of reproductive propagules.

Taxonomy

Zasmidium cassines Kharwar, Arch. Singh, Raghv. Singh & Sham. Kumar, **sp. nov.** Fig 1

MycoBank MB 809138

Differs from *Zasmidium elaeodendri* by its verruculose superficial mycelium, shorter, narrower and less septate conidia and conidiophores.

Type: Nepal, Chitwan, Narayanghat, on living leaves of *Cassine glauca* (Rottb.) Kuntze [= *Elaeodendron roxburghii* Wight & Arn.] (*Celastraceae*), December 1993, coll. R.N. Kharwar, (**Holotype**, HCIO 42337; **isotype** GPU 3002).

Etymology: the epithet is derived from the name of the host genus.

Infection spots amphigenous, brown, circular to subcircular, 1–6 mm diam., coalescing and spreading to cover most of the leaf surface. Colonies hypophyllous, effuse. Mycelium internal and external, septate, branched, verruculose, subhyaline to pale brown, up to 3 µm wide. Stromata absent. Conidiophores arising singly as lateral branches of superficial hyphae, semi-macronematous, mononematous, erect to procumbent, straight to flexuous, smooth-walled, brown, 8.5–60 µm × 2–4.5 µm. Conidiogenous cells integrated, polyblastic, terminal or intercalary, sympodial, cylindrical, conidiogenous loci cicatrized with slightly thickened scars. Conidia holoblastic, dry, acropleurogenous, simple to catenate, rarely branched, cylindrical to obclavate, straight to curved, subhyaline to olivaceous brown, verruculose, 0–6-septate, sometimes constricted at septa, base obconicotruncate with slightly thickened hilum, apex acute to subacute, 13.5–109 × 1.5–4µm.

Only one species of *Zasmidium, Z. elaeodendri* (Kamal et al.) Kamal [≡ *Stenella elaeodendri* Kamal et al.] has been previously reported to occur on *Cassine glauca* (as *Elaeodendron roxburghii*; Kamal 2010). *Zasmidium elaeodendri* differs by its lack of verruculose superficial mycelium, its longer and wider

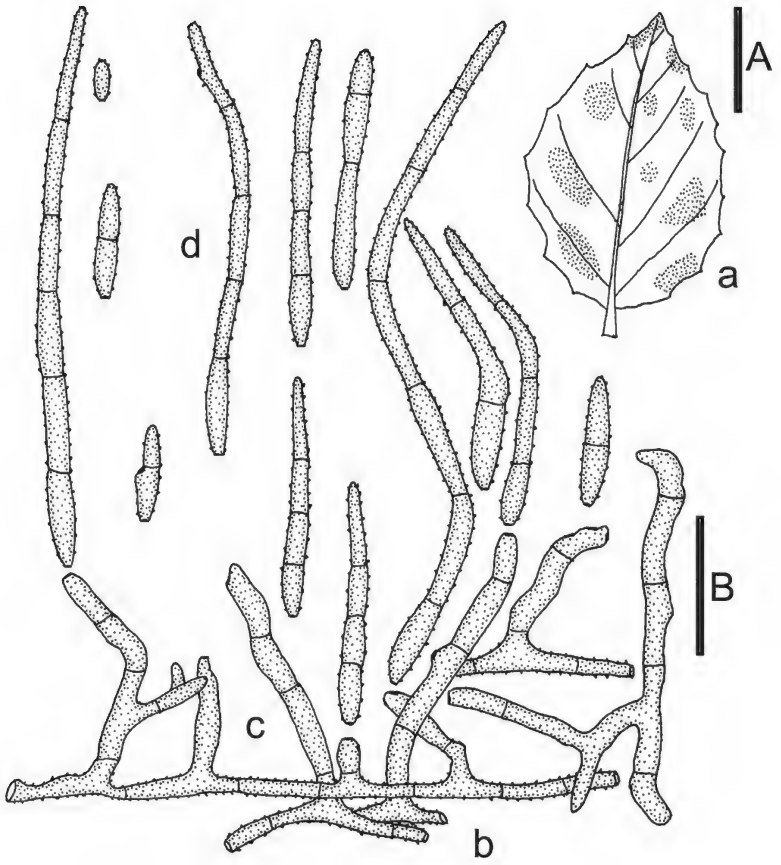

FIGURE 1. *Zasmidium cassines* (HCIO 42337)
a. Infection spots. b. Mycelium. c. Conidiophores. d. Conidia.
Scale bars:A= 20 mm, B = 20 μm

branched conidiophores (≥126 × 4.5–6 μm), and its much longer and thicker 17-septate conidia (≥132 × 3.5–7 μm), which are colourless, smooth-walled, and slightly curved with a conico-truncate base and a rounded apex. The proposed taxon, therefore, merits description as a new species.

Some other cercosporoid hyphomycete genera have been reported on *Cassine: Pseudocercospora elaeodendri* (G.P. Agarwal & Hasija) Deighton on *Cassine glauca* [≡ *Elaeodendron glaucum*] and *Ps. paradoxa* U. Braun & Bagyan. on *Cassine* [= *Elaeodendron*] sp. (Deighton 1976, Braun & Bagyanarayana

1999). *Pseudocercospora* differs from *Z. cassines* in the absence of thickened scars at the conidial bases and on conidiogenous loci.

Kamal (2010: 260–261) listed an erroneous record of *Scolecostigmina tirumalensis* on *Cassine glauca* [≡ *Elaeodendron glaucum*], but this is based on a record of *Stigmella tirumalensis* (Bagyanarayana et al. 1992).

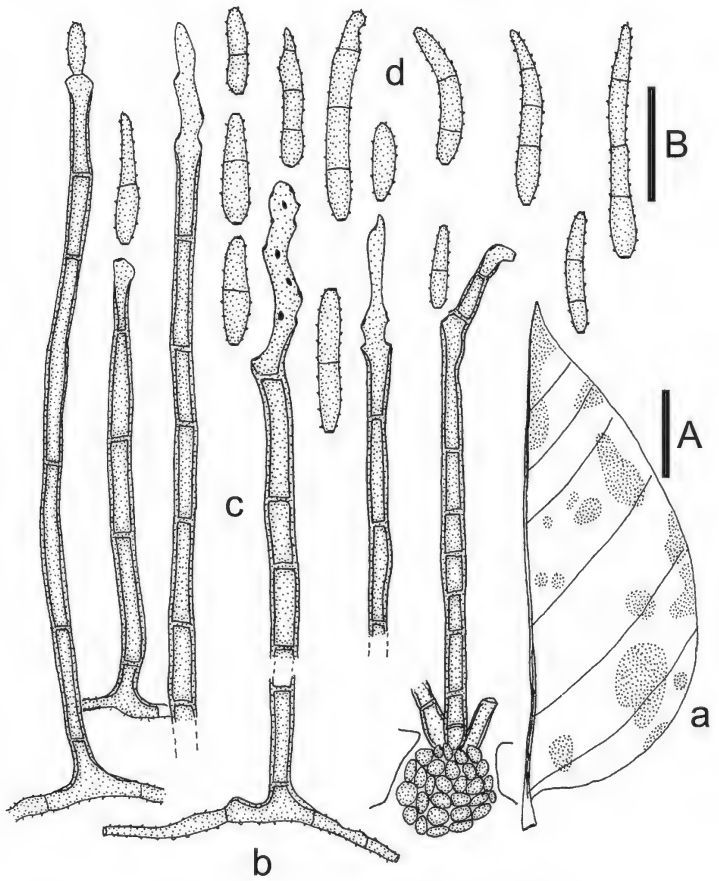

FIGURE 2. *Zasmidium fabaceicola* (HCIO 42338)
a. Infection spots. b. Mycelium.c. Conidiophores.d. Conidia.
Scale bars: A = 20 mm, B = 20 μm

Zasmidium fabaceicola Kharwar, Arch. Singh, Raghv. Singh & Sham. Kumar, **sp. nov.** FIG 2

MYCOBANK MB 809139

Differs from *Zasmidium fabacearum* by its well-developed stromata, and its shorter conidia and conidiophores.

TYPE: Nepal, Chitwan, Narayanghat, on living leaves of *Vigna unguiculata* (L.) Walp. [= *Dolichos biflorus* L.] (*Fabaceae*), January 1994, coll. R.N. Kharwar (**Holotype**, HCIO 42338; **isotype**, GPU 3413).

ETYMOLOGY: the epithet is derived from the host family name.

Infection spots amphigenous, blackish brown, circular to irregular, 1–15 mm diam., later coalescing to form large irregular patches, sometimes covering the entire leaf surface. Colonies hypophyllous, effuse, brown. Mycelium mostly external, septate, branched, verruculose, pale brown, 1.5–3.5 µm wide. Stromata substomatal to superficial, pseudoparenchymatous, dark brown, 17–19 µm in diam. Conidiophores macronematous, arising singly as lateral branches of external hyphae, or in fascicles from stromata, erect, straight to slightly flexuous, unbranched, smooth-walled, mid brown to dark brown below and paler towards the apex, 4–12-septate, 75–210 × 1.5–5 µm. Conidiogenous cells integrated, polyblastic, terminal or intercalary, sympodial, cylindrical, geniculate, scars thickened. Conidia dry, acropleurogenous, simple to catenate, unbranched, cylindrical to obclavato-cylindrical, straight to slightly curved, brown, verruculose, 0–3-septate, sometimes constricted at septa, hilum slightly thickened, base obconico-truncate, apex rounded to obtuse and sometimes cicatrized, 13.5–40 × 1.5–5µm.

Only one species of *Zasmidium,* Z. *fabacearum* (K. Srivastava et al.) Kamal (2010), has been previously reported on *Dolichos*, although the host was not identified to species. *Zasmidium fabacearum* differs from *Z. fabaceicola* by its lack of stromata and by its longer conidiophores (100–310 × 2–4 µm) and conidia (12–60 × 2–6 µm).

Acknowledgments

The authors thank Prof Dr Kamal, DDU Gorakhpur University, Gorakhpur, for helpful taxonomic advice. We are grateful to Dr. Marcin Piątek and Dr Eric H.C. McKenzie for helpful comments and corrections. We also express our deep thanks to Dr Shaun Pennycook for nomenclatural review and Dr Lorelei L. Norvell, Editor-in-Chief, Mycotaxon, for final acceptance of the manuscript for publication. Last but not least, Dr Archana Singh thanks Department of Science and Technology (DST), Government of India, New Delhi for providing financial support under Women Scientist Scheme (WOS-A).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.247

Volume 130, pp. 247–251 *January–March 2015*

The lichen genus *Leiorreuma* in China

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Abstract — Four species of *Leiorreuma* (*Graphidaceae, Ostropales*) are reported from China. *Leiorreuma crassimarginatum* is new to science, and *L. dilatatum* and *L. melanostalazans* are new to China. Descriptions accompany a key distinguishing the four *Leiorreuma* species in China. All materials are conserved in HMAS-L.

Keywords — *Ascomycota,* lichenized fungi, *Ostropomycetidae*, taxonomy,

Introduction

According to the revised generic concept (Staiger 2002), *Leiorreuma* Eschw. belongs to the family *Graphidaceae* (*Ostropales*, *Ostropomycetidae*, *Ascomycota*). The lichen genus is characterized by apothecia with conspicuous sessile opened lirellae, a carbonised proper exciple that is often laterally thin but basally well-developed, an inspersed hymenium, and pale brown transversely septate or muriform ascospores that are I–. Only one species, *L. sericeum*, has previously been reported from China. We report here on three additional species for the country, one of which we describe as new to science.

Materials & methods

The specimens examined are deposited in the Herbarium Mycologicum Academiae Sinicae – Lichenes, Beijing, China (HMAS-L). A dissecting microscope (Tech XTS-30D with Canon 600D camera) and a light microscope (Olympus SZ-51) were used for the morphological and anatomical studies. Measurements and illustrations were taken from the manual cross-sections of fruit bodies in tap water. The lichen substances were detected and identified by thin-layer chromatography (TLC) (Culberson & Kristensson 1970, Culberson 1972, Orange et al. 2001).

Taxonomy

Leiorreuma crassimarginatum Z.F. Jia, **sp. nov.** Pl. 1

Fungal Name FN570068

Differs from *Leiorreuma hypomelaenum* by its production of norstictic acid and its larger ascospores.

Type: China. Guizhou Province, Tongren City, Mt. Fanjing, Daling, 27°55′N 108°41′E, alt. 1570 m, on bark, 5/X/2004, coll. Jiang-Chun Wei & Tao Zhang G425 (**Holotype**, HMAS-L 071776).

Etymology: Latin *crassus* and *marginatus*, in reference to the thick thalline margin.

Thallus corticolous, crustose, grey to pale white, rough, thin, tightly attached to the substratum, without isidia and soralia. Apothecia lirelliform, short to elongate, open, simple or sometimes branched, 1–3 mm long, 0.3–0.6 mm wide, sessile, with thick lateral thalline margin, often rounded at the ends, not striate, scattered over the thallus; disc opened, pale brown; proper exciple laterally slightly and basally thickly carbonized, basal exciple 90–130 µm high; epithecium brownish, 2–5 µm high; hymenium brownish, inspersed, 85–130 µm high, I–; hypothecium brown, 15–40 µm high; paraphyses simple, 1–1.5 µm wide, apices unbranched; asci cylindrical to clavate, 6-spored, 80–120 × 15–25 µm; ascospores brown to brownish, thick-walled, ellipsoid, transversely septate, often 7–12-locular, 35–45 × 10–15 µm.

Chemistry: C–, K+ yellow to brown, P+ yellow; norstictic and stictic acids detected by TLC.

Distribution & ecology: Known only from the type locality, Mt. Fanjing, an important nature reserve in Guizhou Province, South China. Other lichens growing in the surrounding area are species of *Graphis*, *Diorygma*, and *Ocellularia*.

Comments: *Leiorreuma crassimarginatum* is characterized by its sessile lirellae with thick lateral thalline margins, opened discs, entire labia, thickened carbonized basal exciples, the small, brown and thick-walled ascospores, and the presence of norstictic and stictic acids. The new lichen is similar to *L. hypomelaenum*, which differs in its smaller (22–38 × 7–11 µm), 7–12-locular, and thin-walled ascospores and its lack of norstictic acid (Staiger 2002).

Leiorreuma dilatatum (Vain.) Staiger, Biblioth. Lichenol. 85: 296 (2002).

Thallus corticolous, crustose, pale white to grey, rough to smooth, thickish. Apothecia lirelliform, short to elongate, open, simple or branched, 0.5–3 mm long, 0.3–0.7 mm wide, sessile, with thin thalline margin; disc opened, black to brown; proper exciple laterally slightly and basally thickly carbonized, basal exciple 30–45 µm high; epithecium brownish, 2–5 µm high; hymenium brownish, inspersed, 80–100 µm high, I–; hypothecium brown, 10–20 µm

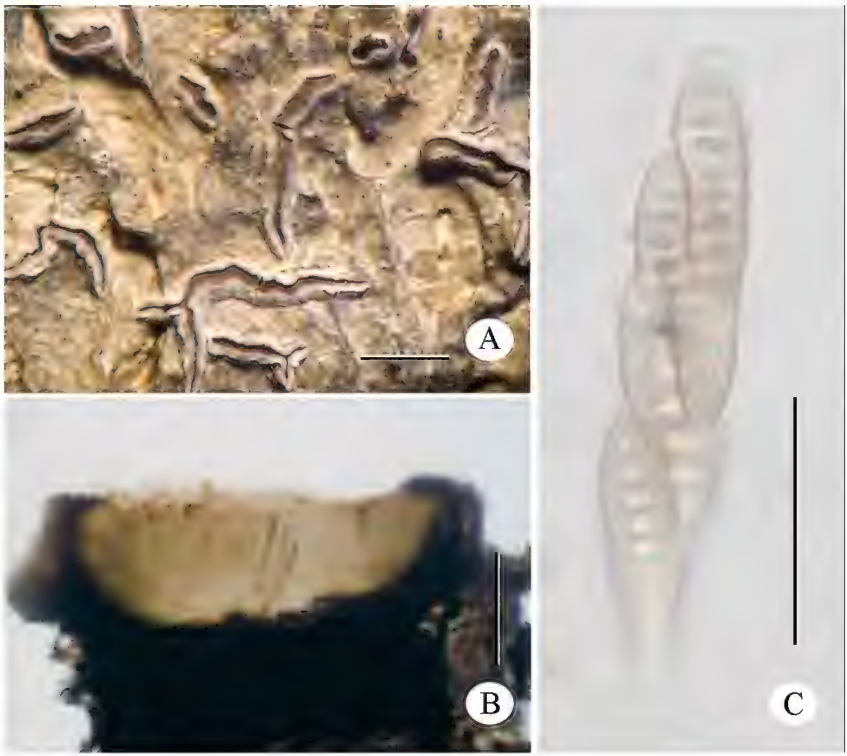

Plate.1. *Leiorreuma crassimarginatum* (holotype, HMAS-L 071776) A. Thallus with lirellae. B. Apothecium, cross-section. C. Ascus containing 6 ascospores. Scales: A = 1 mm; B = 100 µm; C = 50 µm.

high; paraphyses simple, 1–1.5 µm wide, apices unbranched; asci cylindrical to clavate, 8-spored; ascospores brown to brownish, ellipsoid, transversely septate, often 4-locular, 20–30 × 6–8 µm, I+ brown.

Chemistry: C–, K–, P–; no lichen substances detected by TLC.

Distribution & ecology: Collected on Mt. Jianfengling, the largest remaining tropical rainforest on Hainan Island, South China. Associated lichens included species of *Graphis*, *Hemithecium*, and *Lecanora*.

Specimen examined: **China. Hainan Province**, Mt. Jianfengling, alt. 180 m, on bark, 1/X/2008. Jing Li HN081374 (HMAS-L 127475).

Comments: *Leiorreuma dilatatum* is characterized by sessile lirellae with thin lateral thalline margins, opened discs, a basally carbonized exciple, small brown ascospores, and the absence of lichen substances. It is similar to

L. sericeum, which differs by its well-developed thalline and proper margins and its smaller ascospores (14–20 × 5–8 µm, Staiger 2002; 16–18 × 5.5–7.5 µm, our specimens). This is the first report of *L. dilatatum* from China.

Leiorreuma melanostalazans (Leight.) A.W. Archer, Telopea 11: 75 (2005).

Thallus corticolous, crustose, pale grey, rough to smooth, thin. Apothecia lirelliform, short to elongate, open, simple or branched, 1–4 mm long, 0.3–0.5 mm wide, sessile, with thin thalline margin; disc opened, brown; proper exciple inconspicuous, laterally slightly and basally thickly carbonized, basal exciple10–30 µm high; epithecium brownish, 2–5 µm high; hymenium brownish, inspersed, 90–110 µm high, I–; hypothecium brown, 10–15 µm high; paraphyses simple, 1–1.5 µm wide, apices unbranched; asci cylindrical to clavate, 8-spored; ascospores brown to brownish, ellipsoid, transversely septate, often 7–9-locular, 25–40 × 8–10 µm, I+ brown.

Chemistry: C–, K+ yellow, P+ yellow; stictic acid detected by TLC.

Distribution & ecology: Known from two localities in the tropical rainforest in Hainan Island, South China. Associated lichens included species of *Graphis*, *Hemithecium*, and *Phaeographis*.

Specimens examined: **China. Hainan Province**, Mt. Jianfengling, alt. 630–700 m, on bark, 1/X/2008, Jing Li HN081422 (HMAS-L 117057); 30/IX/2008, Ze-Feng Jia HN080683 (HMAS-L 117056).

Comments: *Leiorreuma melanostalazans* is characterized by sessile lirellae with thin lateral thalline margins, opened discs, a basally carbonized exciple, small brown 7–9 locular ascospores, and the presence of stictic acid. It is similar to *L. exaltatum*, which differs by its lack of lichen substances (Staiger 2002). This is the first report of *L. melanostalazans* from China.

Leiorreuma sericeum (Eschw.) Staiger, Biblioth. Lichenol. 85: 305 (2002).

Thallus corticolous, crustose, pale yellow to grey, smooth, thin. Apothecia lirelliform, short to elongate, open, simple or branched, 0.5–3.5 mm long, 0.2–0.3 mm wide, sessile, with thin thalline margins, disc opened, brown with white-grey pruina; proper exciple laterally thinly and basally thickly carbonized, basal exciple 20–40 µm high; epithecium brownish, 2–5 µm high; hymenium brownish, inspersed, 70–90 µm high, I–; hypothecium brown, 10–15 µm high; paraphyses simple, 1–1.5 µm wide, apices unbranched; asci cylindrical to clavate, 8-spored; ascospores brown to brownish, ellipsoid, transversely septate, often 4-locular, 16–18 × 5.5–7.5 µm, I+ purple-brown.

Chemistry: C–, K–, P–; no lichen substances detected by TLC.

Distribution & ecology: Distributed throughout tropical and subtropical South China. Associated lichens included species in the *Graphidaceae* and *Parmeliaceae*.

Specimens examined: **CHINA. Hainan Province**, Mt. Jianfengling, alt. 980 m, 28/VII/2009, Jing Li HN247-1 (HMAS-L 117025). **Guangxi Province**, Shangsi County, alt. 600 m, on bark, 31/XII/1997, Shou-Yu Guo 1638 (HMAS-L).

Comments: *Leiorreuma sericeum* is characterized by sessile lirellae with thin lateral thalline margins, opened discs, a laterally and basally carbonized exciple, small (≤18 μm long) brown 4-locular ascospores, and the absence of lichen substances. It is similar to *L. dilatatum*, which differs by its larger ascospores and poorly developed thalline and proper margins (Staiger 2002). *Leiorreuma sericeum* has previously been reported (as *Phaeographis sericea*) from Hong Kong (Thrower 1988, Wei 1991).

Key to species of *Leiorreuma* known from China

1 Ascospores 4-locular; lichen substances absent 2
1 Ascospores >6-locular; lichen substances present 3
2 Thalline and proper margins poorly developed;
ascospores 20–30 × 6–8 μm ..... ***L. dilatatum***
2 Thalline and proper margins well developed;
ascospores 16–18 × 5.5–7.5 μm ..... ***L. sericeum***
3 Norstictic and stictic acids present; ascus 6-spored;
ascospores 7–12-locular, 35–45 × 10–15 μm ..... ***L. crassimarginatum***
3 Stictic acid present; ascus 8-spored;
ascospores 7–9-locular, 25–40 × 8–10 μm ..... ***L. melanostalazans***

Acknowledgments

This research was supported by the National Natural Science Foundation of China for the funds (No. 31270066 & 31093440) and Excellent Youthful Scientist Foundation Project of Shandong Province (No. BS2011SW028). The authors are grateful to Dr. Santosh Joshi (CSIR-National Botanical Research Institute, Lucknow, India) and Hua-Jie Liu (College of Life Sciences, Hebei University, China) for their valuable comments on the manuscript and for acting as presubmission reviewers.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.253

Volume 130, pp. 253–258 *January–March 2015*

Periconiella liquidambaricola sp. nov. – a new Chinese hyphomycete

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Abstract — The new species *Periconiella liquidambaricola*, collected on living leaves of *Liquidambar formosana* (*Altingiaceae*) in Jiangxi Province, China, is described, illustrated and compared with other species of the genus *Periconiella*. The new fungus is the first species described from a host belonging to the *Altingiaceae*. *Periconiella liquidambaricola* is well characterized by its very long conidiophores and smooth, pale scolecosporous conidia. It has similar conidia to *Periconiella lygodii* on fronds of *Lygodium flexuosum* in India, but differs in having conidia with attenuated to acute apexes and much longer conidiophores with uninflated bases.

Key words — *Ascomycota*, asexual morph, *Periconiella qualeicola*, Asia

Introduction

Leaves of *Liquidambar formosana* infected by a range of plant pathogenic micromycetes were recently collected during a biodiversity-ecosystem study in a subtropical forest near Xingangshan, Jiangxi Province, China. One of these fungi had conidiophores with repeatedly and densely branched apices, pigmented conidiophores with several conspicuous conidiogenous loci, and solitary, holoblastically formed conidia that was readily identifiable as a species of the genus *Periconiella* Sacc. This fungus represents the first collection of a *Periconiella* species on a host in the *Altingiaceae*. A comparison with other species assigned to *Periconiella* revealed that the Chinese collections represent an undescribed species.

Materials & methods

Light microscopy (Olympus BX50) was used to examine the material (unstained samples mounted in distilled water, oil immersion, 1000×). The ranges of sizes for conidiophores, conidia and other structures were based on 30 measurements. Digital

images were made with a Zeiss Axioskop 2 with Zeiss AxioCam HR and occasionally optimized with the software Zeiss AxioVision. Freehand drawings were made on the basis of microscopic preparations.

Taxonomy

Periconiella liquidambaricola U. Braun, S. Bien & Hönig, **sp. nov.** Figs. 1, 2

MycoBank MB809832

Periconiellae lygodii *morphologice similis sed maculis foliorum nullis, conidiophoris multo longioribus, ad basim non inflatis et conidiis apice attenuato vel acuto.*

Type: China, Jiangxi Province, Xingangshan, subtropical forest site of the BEF-China Project, 29.1250° N, 117.9085° E, on living and wilting leaves of *Liquidambar formosana* (*Altingiaceae*), 8 Sep. 2013, coll. S. Bien (**Holotype**-HAL 2673 F).

Etymology: Derived from the name of the host genus (inhabitant of *Liquidambar*).

Leaf spots indistinct or lacking. Colonies on living green leaves or on diffuse, discolored patches of wilted leaves, yellow or ochraceous to pale brown. Mycelium internal and external; superficial hyphae emerge through stomata, branched, straight to sinuous, 1–4 µm wide, septate, subhyaline to medium brown, thin-walled, verruculose. Stromata not developed. Conidiophores solitary, scattered, arise from superficial hyphae, at the top of mother cells, erect, straight to slightly curved, 400–700 µm long, unbranched stalk 5–10 µm wide, not swollen at the base, pluriseptate throughout, distance between septa 25–50 µm, dark brown throughout or somewhat paler towards the apex, wall thickened, 1–2 µm, smooth, apical portion 2–5 times densely forked, branched part 50–100 µm long, primary branches about 25–70 µm long, subcylindrical. Conidiogenous cells integrated, terminal, 10–30 µm long and 3–6 µm wide, subcylindrical or enlarged (subclavate), up to 9 µm, straight to moderately geniculate-sinuous, with a single or several conspicuous conidiogenous loci,

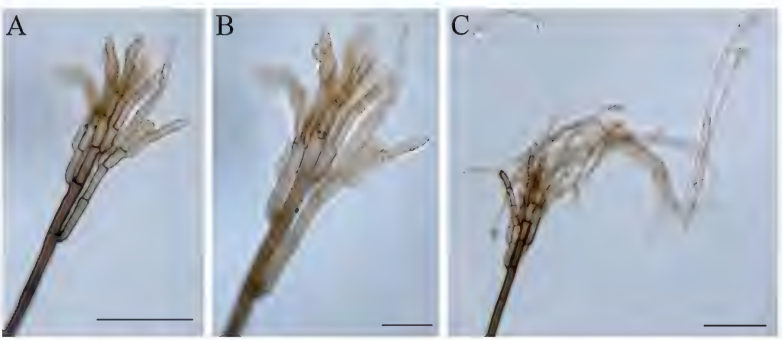

Fig. 1. *Periconiella liquidambaricola*. Micrographs of branched apical portions of conidiophores: A. focussed on outline; B. focussed on conidiogenous loci; C. with adherent and detached conidia. Bars: A, C = 50 µm; B = 20 µm.

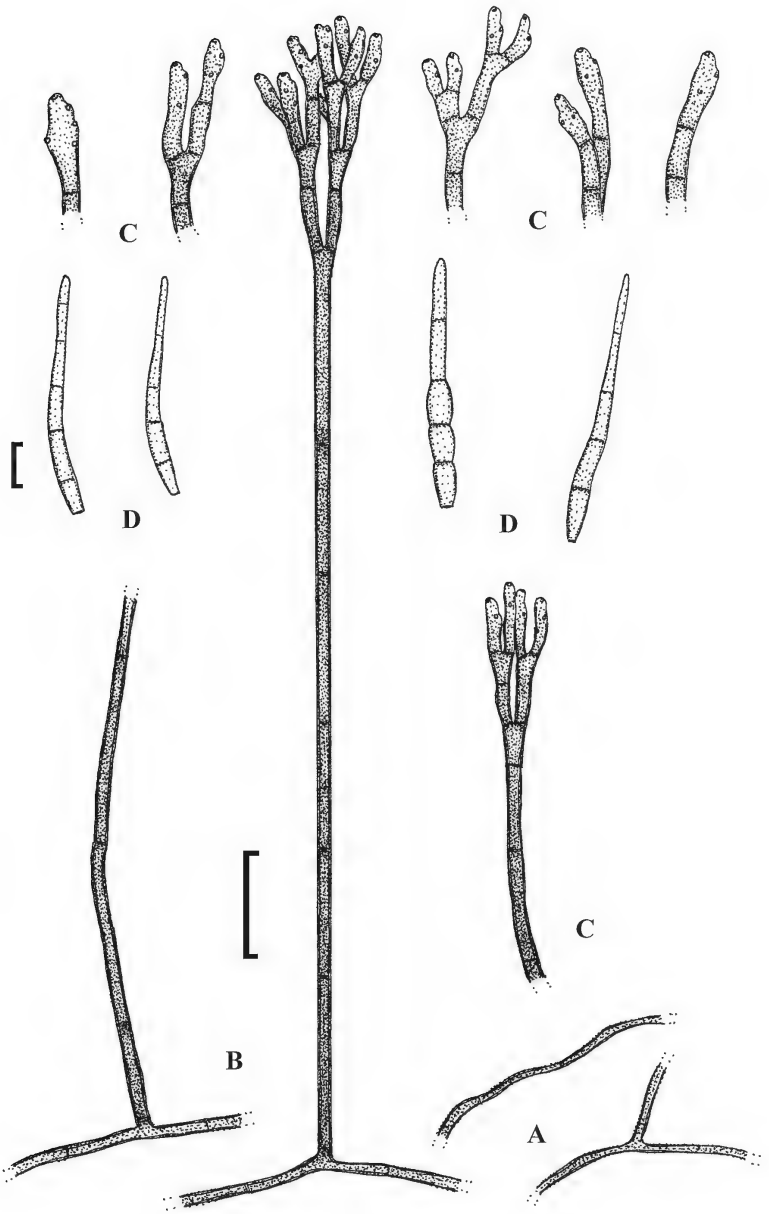

Fig. 2. *Periconiella liquidambaricola*.
A. superficial hyphae; B. solitary conidiophores arising from superficial hyphae;
C. branched apical parts of conidiophores; D. conidia. Bars: B, C. = 50 μm; D = 10 μm.

circular, ring-like, 1.5–3 µm diam, rim darkened-refractive, barely or slightly thickened. Conidia solitary, obclavate, attenuated towards the tip, 50–140 × 4–7.5 µm, 3–7-septate, sometimes constricted at the septa, pale brown, thin-walled, smooth, apex subobtuse to pointed, base short obconically truncate, hila 2–2.5 µm wide, darkened-refractive, at least around the rim.

Discussion

Arzanlou et al. (2007) published a modern description of *Periconiella* and revealed its phylogenetic affinity within the *Mycosphaerellaceae*. *Periconiella velutina* (G. Winter) Sacc., the type species, together with most other species assigned to *Periconiella* are characterized by having amero-, didymo- or phragmosporous conidia (Ellis 1967, 1971, 1976; Priest 1991; Hosagoudar & Braun 1995; McKenzie 1996; Arzanlou et al. 2007; Mafia et al. 2008, etc.). All known species of this genus are specialized and confined to hosts in particular genera of plant families. *Periconiella liquidambaricola* is the first species described from a host in the *Altingiaceae*.

The conidial shape places *P. liquidambaricola* in a small group of species characterized by scolecosporous conidia. *Periconiella lygodii* Arch. Singh et al. ex U. Braun (Braun 2004), described from India on *Lygodium flexuosum*, is a morphologically similar species. A record of "*Periconiella* sp. close to *P. rapaneae* M.B. Ellis" on *Lygodium flexuosum* from Myanmar (Thaung 2008) seems to belong to *P. lygodii*. Additional records of *P. lygodii* are known from the Philippines (Begum et al. 2009) and Taiwan (Kirschner & Chen 2010), in both cases on *Lygodium japonicum*, and from Laos on *L. polystachyum* (Phengsintham et al. 2013). *Periconiella lygodii* is characterized by similar smooth scolecosporous conidia, but forms distinct leaf spots, much shorter conidiophores that are enlarged at the base, and conidia that are rounded at the apex (Braun 2004).

Periconiella rapaneae M.B. Ellis on *Rapanea* in Ecuador (Ellis 1967, 1971) is morphologically close to *P. lygodii* but differs in having long primary branches (to 170 × 5–8 µm). *Periconiella araliacearum* G.F. Laundon (Laundon 1972), *P. arcuata* Arzanlou et al. (Arzanlou et al. 2007), *P. longispora* Kamal et al. (Kamal et al. 1979), *P. maianthemi* R. Kirschner (Kirschner & Piepenbring 2008) and *P. santaloidis* M.B. Ellis (Ellis 1967, 1971) are similar species. *Periconiella araliacearum* differs in having much shorter conidiophores (to 200 µm) with sparingly branched apices, and long, verruculose conidia (to 170 µm). *Periconiella longispora*, described from India on *Litsea chinensis*, is characterized by having long conidiophores (to 800 µm) formed in fascicles, with branches up to 175 µm, and rather broad (36–180 × 4.5–9.5 µm), smooth and 3–20-septate conidia. *Periconiella arcuata* is distinct with its falcate, verruculose conidia, and *P. maianthemi* is distinguishable by having much shorter conidiophores

(200–435 µm) and shorter verruculose conidia, (13.5–)19–43(–75) µm. *Periconiella santaloidis* differs in having conidiophores with densely branched apices, short branchlets and verruculose conidia.

An additional comparable species is:

Periconiella qualeicola Dorn.-Silva & Dianese, **nom. nov.**

MycoBank MB809833

≡ *Periconiella longispora* Dorn.-Silva & Dianese, Mycologia 95: 1246, 2004 ["2003"], nom. illeg.,
non *P. longispora* Kamal, Suj. Singh & R.P. Singh,1979.

This leaf-spotting hyphomycete described from Brazil (Dornelo-Silva & Dianese 2004), can be distinguished by its much wider cylindrical conidia (37–95 × 5–8 µm).

Periconiella liquidambaricola seems to be rather common in the subtropical forest site near Xingangshan, Jiangxi Province. Numerous leaf samples of *Liquidambar formosana* were collected for mycological examination. Mycelium with pigmented verruculose hyphae assignable to *P. liquidambaricola* has been found on most leaves, but well developed conidiophores have been observed on a limited number of leaves.

Acknowledgments

We gratefully acknowledge funding by the German Research Foundation (DFG, FOR 891/1 and 891/2) and the National Science Foundation of China (NSFC, 30710103907 and 30930005). We are also much obliged to V.A. Mel'nik (Russia) and R.G. Shivas (Australia) for pre-submission reviews.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.259

Volume 130, pp. 259–264 *January–March 2015*

First record of *Erysiphe syringae-japonicae* in Turkey

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Abstract — *Erysiphe syringae-japonicae* was reported on leaves of *Syringa vulgaris* for the first time from Turkey. A short description, distribution, and illustrations for this powdery mildew fungus are provided and discussed briefly.

Key words — Asia Minor, *Erysiphales*, invasive species, lilac, *Microsphaera*

Introduction

A powdery mildew collected in Japan on the lilac, *Syringa amurensis* var. *japonica* [= *S. reticulata*], was described by Braun (1982) as *Microsphaera syringae-japonicae* (*Erysiphales*, *Ascomycota*). Later, this species was reported from the Russian Far East (Bunkina 1991, as "*Microsphaera syringae*") and from Korea (Shin 2000). *Microsphaera syringae-japonicae* was already known on lilacs in North America and Europe, and was distinguished from *M. syringae*, mainly by its evanescent mycelium, its larger number of spores in the ascus, and its usually more extensively pigmented chasmothecial appendage bases.

In 1988, one of the authors (VP Heluta) critically examined the type specimens of powdery mildews described from the Russian Far East, and showed that one of them, the type specimen of *M. aceris* on leaves of *Acer barbinerve,* had chasmothecia identical to those of *M. syringae-japonicae*. However they were in adherent groups that could have drifted from another host such as a *Syringa* sp. Thus, *M. aceris* is an earlier synonym for *M. syringae-japonicae* and has priority within the genus *Microsphaera* Lév. However, as already discussed by Braun et al. (2003) and Braun & Cook (2012), *Microsphaera* is now considered a section of the genus *Erysiphe* DC.; as the epithet *aceris* is already occupied in *Erysiphe*, the correct current name is *E. syringae-japonicae* [≡ *M. syringae-japonicae*].

In the 1990s, *E. syringae-japonicae* from East Asia was introduced into Europe (Seko et al. 2008, 2011), where it is now common in Germany, Poland, Russia, Ukraine, Switzerland, etc. (Braun 1998, 2002, Piątek 2003, Heluta 2006, Rusanov & Bulgakov 2008, Braun & Cook 2012). However, it had not been reported in either the European or Asian parts of Turkey until autumn 2012, when one of the authors (I. Akata) collected it on *Syringa vulgaris* in Ankara.

Materials & methods

Chasmothecia were studied on herbarium material dried between papers at 22–24°C. They were examined in distilled water and photographed under a light microscope «Primo Star» (Carl Zeiss, Germany) using the Camera «Canon A 300» and software «AxioVision 4.7». Digital data were analysed statistically with n ≥30 for each character. For study under the scanning electron microscope (JSM–6060LA, Japan), dried pieces of infected *Syringa* leaves were glued onto the metal stubs and coated with gold.

Identification was made using the descriptions of Braun & Cook (2012). In addition, the specimen was compared with samples of *E. syringae-japonicae* collected in Germany, Switzerland, Ukraine, and the Russian Far East. The studied materials are deposited in the National Herbarium of M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev, Ukraine (KW).

Taxonomy

Erysiphe syringae-japonicae (U. Braun) U. Braun & S. Takam., Schlechtendalia 4: 14. 2000. Figs 1, 2

≡ *Microsphaera syringae-japonicae* U. Braun, Mycotaxon 15: 121. 1982.

= *Microsphaera aceris* Bunkina, Komarovskie Chteniya 21: 82. 1974 [non *Erysiphe aceris* DC. 1815].

≡ *Erysiphe acerina* U. Braun & S. Takam., Schlechtendalia 4: 5. 2000.

Mycelium amphigenous, white, often effused or in patches, evanescent to sub-persistent. Hyphae thin, up to 7 µm wide. Hyphal appressoria well developed, lobed, in opposite pairs or sometimes single. Chasmothecia brown to dark brown, scattered to gregarious on the leaf surface, 70–90 µm diam. Peridium cells polygonal to almost rounded, 10–16 × 18–21 µm. Appendages 4–12, equatorial, aseptate, stiff, usually straight to rarely curved, smooth to rough, thick-walled, hyaline but brownish or brown at the base, 75–130 µm long, apices 4–5 times regularly dichotomously branched often in three dimensions (i.e., branched part not fully flat), rather compact, sometimes primary and secondary branches elongated and therefore apical parts somewhat deeply forked, tips recurved. Asci 3–6, sessile or short-stalked, broadly ellipsoid or slightly ovoid, hyaline, 40–55 × 30–40 µm, (5–) 6–8-spored. Ascospores hyaline, ellipsoid or ovoid, 16–22 × 11–14 µm.

Specimen examined — **TURKEY, Ankara**, Tandogan, 39°56′N 32°49′E, alt. 860 m, on *Syringa vulgaris* L. (*Oleaceae*), 17.10.2012, Ilgaz Akata (KW 40328F).

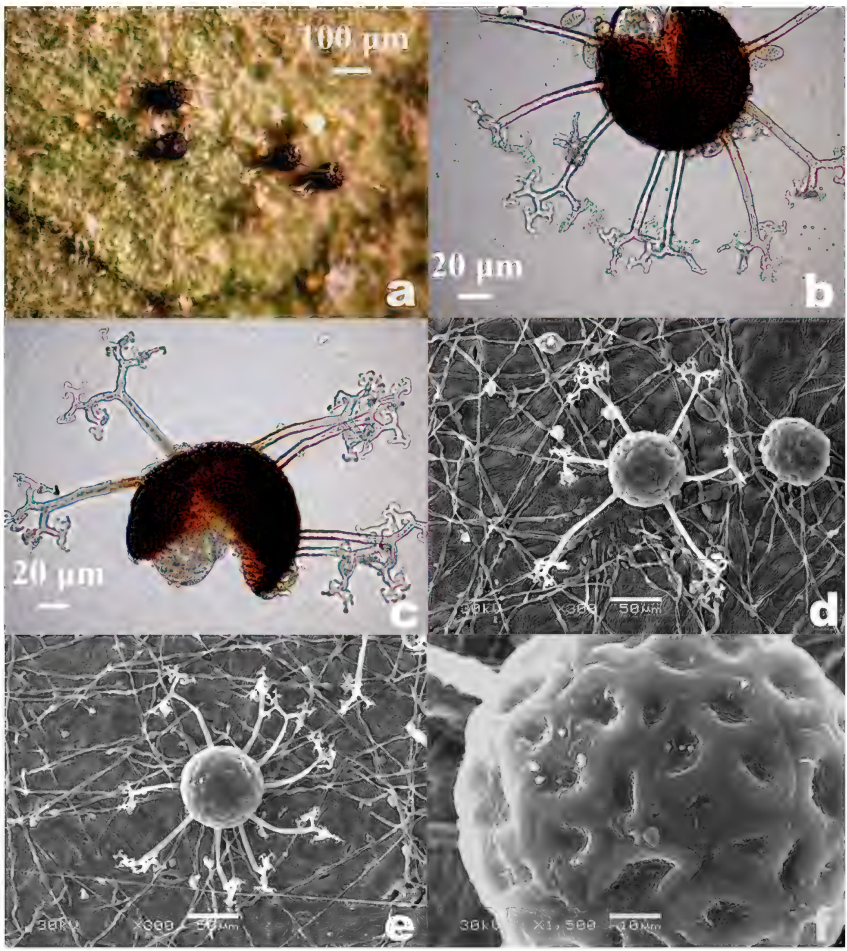

FIG. 1. *Erysiphe syringae-japonicae* (KW 40328F) on *Syringa vulgaris*: **a** – chasmothecia on the host plant leaf (upper surface, in reflected light); **b, c** – chasmothecia splitting with release of ascospores (**b**) and asci (**c**) and with appendages colored at the base; **d, e** – chasmothecia with appendages (SEM); **f** – surface of the peridium (SEM).

Discussion

Many *Syringa* species are cultivated in different countries of the northern hemisphere as important ornamental plants. Some of them are susceptible to powdery mildew caused by *Phyllactinia fraxini* (DC.) Fuss and two species of *Erysiphe* sect. *Microsphaera*. *Phyllactinia fraxini* is uncommon and causes little harm to its hosts. The other two species, *E. syringae* and *E. syringae-japonicae*,

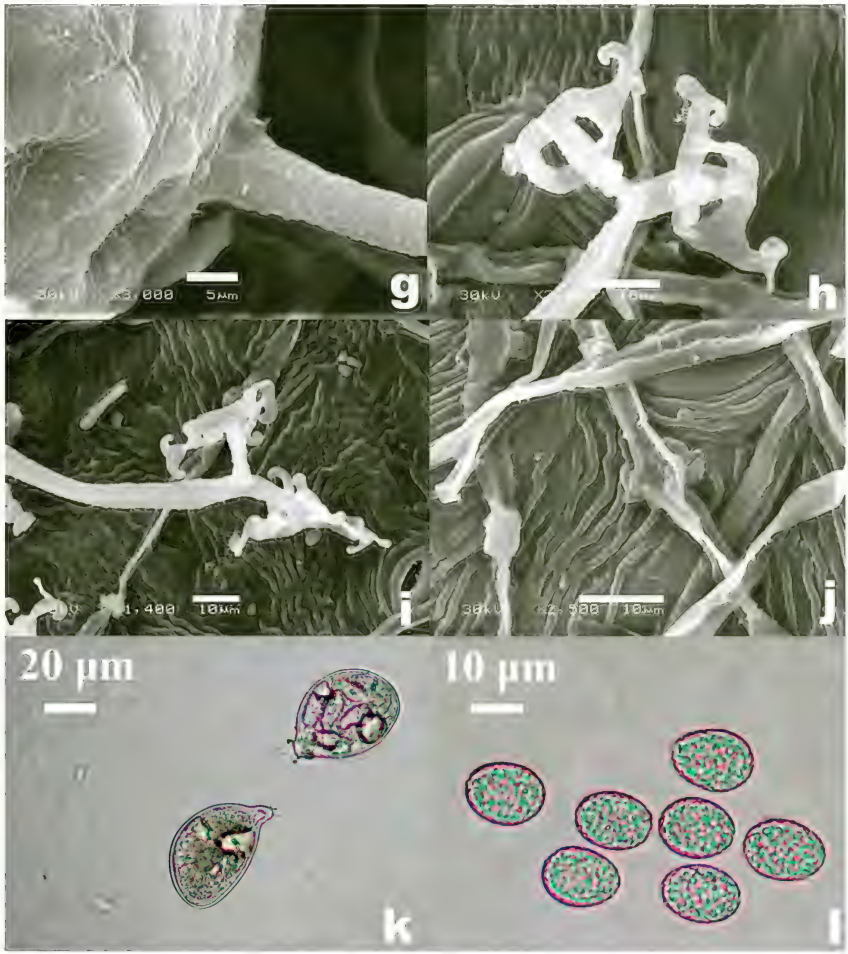

Fig. 2. *Erysiphe syringae-japonicae* (KW 40328F): **g** – basal part of chasmothecial appendage (SEM); **h, i** – apical parts of appendages (SEM); **j** – paired hyphal appressoria (SEM); **k** – asci; **l** – ascospores.

are common, and *E. syringae-japonicae* especially can harm the hosts. Seko et al. (2008, 2011) studied the worldwide spread of these fungi and found the origin of *E. syringae* to be North America from whence it was introduced to Europe, East Asia, and South America. However, recently the fungus has been supplanted by the Asian *E. syringae-japonicae,* particularly in Europe. The last time *E. syringae* was collected in Ukraine was in 2003 (Seko et al. 2011), where it formed only the anamorph.

Erysiphe syringae and *E. syringae-japonicae* are different genetically (only 94% similarity between the ITS sequences of the two species; see Seko et al. 2008) as well as morphologically. *Erysiphe syringae* has typically 4–5-spored asci and nearly colorless appendages, while the asci of *E. syringae-japonicae* contain (5–)6–8 spores and its appendages are more or less pigmented from their base up to the middle part. Thus, these species can be easily distinguished by morphological analysis.

Lilac (*Syringa vulgaris*) is also cultivated in Turkey as an ornamental plant. According to the recent literature on Turkish powdery mildews in Turkey, only *E. syringae* has been previously reported on this plant (Bahcecioglu & Yildiz 2005; Bahcecioglu et al. 2006; Kabaktepe & Bahcecioglu 2006, 2009; Kavak 2011; Severoglu & Ozyigit 2012), and there is no record of *E. syringae-japonicae*. However, our sample is identical to the specimens collected in Europe, especially in Ukraine. Thus, the spread of *E. syringae-japonicae* in Turkey needs more research and monitoring.

Acknowledgments

The authors are grateful to Uwe Braun (Germany) for kindly sending several specimens of *E. syringae-japonicae* collected in West Europe and for his pre-submission review. We thank VI Sapsay (Ukraine) for his help with scanning electron microscopy. We also thank Uwe Braun, Roger Cook, Wieslaw Mulenko, and Shaun Pennycook for their helpful comments and careful review of this article.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.265

Volume 130, pp. 265–273 *January–March 2015*

AFLP characterization of three argentine *Coprotus* species

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Abstract — AFLP methodology was applied to characterize three *Coprotus* species (*C. lacteus, C. niveus, C. sexdecimsporus*) so as to estimate the levels of polymorphism within species, to analyze the phenetic relationships among them, and to contrast the AFLP findings to those of a previous RAPD study. The high number of AFLP bands obtained with the six assayed primers allowed us to detect intra-specific variability. The genetic variability within species obtained using AFLP (measured in terms of percentage of polymorphic loci) was two to three times higher than those obtained by RAPD. The phenograms generated by AFLP markers grouped all strains of the same species into three defined clusters, and a higher association between *C. lacteus* and *C. sexdecimsporus* was also observed. The AFLP technique could become a powerful tool for genera such as *Coprotus*, in which a high intra-specific homogeneity does not allow detection of genetic variability using other PCR-based markers.

Key words — *Ascomycota*, molecular markers, coprophilous

Introduction

The fungal genus *Coprotus* Korf & Kimbr. comprises coprophilous discomycetes, which belong to the *Pyronemataceae* Corda. Species of this genus are homothallic, probably due to an adaptation to the substrate, which determines their sexual isolation (Wicklow 1981). Sexual reproduction produces ascospores dispersed by effective discharge methods.

A homothallic mating system can be very difficult to distinguish from clonality using some molecular markers, since both systems produce progenies that do not show evidence of segregation for these markers and yield linkage disequilibrium at the population level over the long term (Billiard et al. 2012).

Coprotus species have been traditionally distinguished by such cytological and morphological characters (Kimbrough et al. 1972) as the number of ascospores per ascus, the presence or absence of pigments in paraphyses and excipulum, and the size and shape of asci, ascospores and sterile elements.

Different concepts have been used to define fungal species. The phenotypic concept is the classic approach based on morphology, and *Coprotus* species have traditionally been classified on cytological and morphological characters (Kimbrough et al. 1972). However, difficulties often arise while attempting to identify *Coprotus* species, as they are morphologically very similar, characters frequently overlap, and some vary greatly according to the culture conditions (Dokmetzian et al. 2005). A polythetic concept, which circumscribes species based on a combination of characters (Kohn 1992, Guarro et al. 1999), could prove helpful in characterizing the *Coprotus* genus.

RAPD [random amplified polymorphic DNA] analysis was performed on *Coprotus* species by Ramos et al. (2008). In that study, the RAPD technique confirmed the previous identification of strains using morphological characters (Dokmetzian et al. 2005) and provided a greater number of species-specific bands, but genetic variability within species (in terms of percentage of polymorphic loci) was very low. All strains had the same geographic origin and showed identical patterns for the six primers assayed on the three *Coprotus* species studied. Therefore, it would seem that to detect variability among strains, other PCR markers, such as AFLP, should be used.

The amplified fragment length polymorphism (AFLP) method (Vos et al. 1995) is a DNA-based fingerprinting technique, which requires no prior sequence information on the organism under scrutiny. This method is used to determine the degree of similarity among isolates and offers certain advantages over other techniques, i.e., high level of identified polymorphism, high reproducibility, and relative technical simplicity (Briad et al. 2000). AFLP analysis has been successfully used to estimate the amount of genetic variation within fungal species. Given that a large number of amplicons can be screened, AFLP is especially useful for characterizing clonal lineages and for establishing phenetic relationships among species with little morphological differentiation. AFLP has a clear advantage over other PCR based markers such as RAPDs, not only in terms of reproducibility but also because more loci can be screened in each reaction.

In our present study we applied the AFLP methodology to characterize three *Coprotus* species—*C. lacteus* (Cooke & W. Phillips) Kimbr., *C. niveus* (Fuckel) Kimbr., and *C. sexdecimsporus* (P. Crouan & H. Crouan) Kimbr. & Korf—so as to estimate the levels of polymorphism within species, to analyze the phenetic relationships among them, and to contrast the findings of our AFLP study to those of a previous RAPD study.

Table 1. Fungal strains of *Coprotus* species used, with their Argentinian collection localities and BAFC numbers

Strain	Geographical location *	BAFC
C. lacteus		
lacA1(11), lacA3(12), lacA6(13), lacA10(14), lacA13(15)	Agronomía, CABA	874, 1937, 1940, 1941, 1942
lacL1(16), lacL 3 17), lacL4(18), lacL6 (19)	Villa Lugano, CABA	1944, 1945, 1946, 1947
C. niveus		
nivBC2 (25), nivBC3 (27), nivBC4 (30)	Bahia Craft, Villa La Angostura, NQ	1970, 1971, 1972
nivE1 (48), nivE2 (49)	Bahía Ensenada, TF	1956, 982
nivC2 (42), nivC3 (38), nivC4 (45), nivC5 (46)	Campana, PBA	1960, 1961, 1962, 1963
nivU1 (36), nivU3 (37), nivU6 (39), nivU7 (40), nivU8 (41)	Ciudad Universitaria, CABA	1964, 1965, 1966, 1967, 1968
nivL1 (34), nivL3 (35)	Villa Lugano, CABA	1973, 1974
C. sexdecimsporus		
sexU1 (6), sexU2 (7), sexU4 (8)	Ciudad Universitaria, CABA	1952, 1953, 1954
sexG1 (5), sexG4 (21), sexG7 (23)	Los Gigantes, C	1948, 1950, 1951

* C = Córdoba province:, CABA = Buenos Aires city; NQ = Neuquén province; PBA = Buenos Aires province; TF = Tierra del Fuego province.

Materials & methods

Isolation and maintenance of monosporic strains

The characters used to identify the *Coprotus* species were those used by Kimbrough et al. (1972). The isolation and maintenance of monosporic strains followed the procedure indicated by Suárez et al. (2006). Thirty-one monosporic strains, from nine geographical locations, were used (Table 1). All strains were deposited in the Herbarium and Culture Collection of the Department of Biodiversity, Faculty of Natural & Exact Sciences, University of Buenos Aires, Argentina (BAFC).

DNA extraction

Mycelium was obtained as in Ramos et al. (2000) and ground to powder in liquid nitrogen using a sterile pestle. Genomic DNA was extracted following Gottlieb & Lichtwardt (2001). Quality control and quantification of genomic DNA was carried out by agarose gel (0.8% w/v) electrophoresis and by comparison with a DNA molecular-size standard (Lambda EcoRI/HinDIII, Promega Corp.). Ethidium bromide gels were photographed under UV light.

AFLP

The AFLP methodology was carried out on 250 ng of genomic DNA using the AFLP® Analysis System for Microorganisms Primer Kit (Invitrogen) as described in the instructions manual with minor modifications (Gottlieb et al. 2005). Selective primers were combined as in Table 2.

Table 2. AFLP primers used in this study

Primers	Sequence 5′-3′
E+ACG	GAC TGC GTA CCA ATT CAC G
E+AAC	GAC TGC GTA CCA ATT CAA C
E+AAG	GAC TGC GTA CCA ATT CAA G
M+A	GAT GAG TCC TGA GTA AA
M+G	GAT GAG TCC TGA GTA AG
M+C	GAT GAG TCC TGA GTA AC
M+T	GAT GAG TCC TGA GTA AT

* E stands for *EcoRI* and M stands for *MseI*.

All PCR amplifications were performed in a TECHNE PROGENE thermal cycler. Polyacrylamide gel electrophoresis conditions followed Gottlieb et al. (2005). A 30–330 bp AFLP® DNA Ladder (Invitrogen) size marker was included twice in each electrophoresis, and the size of AFLP bands scored ranged from 90 to 330 bp. AFLP bands were visualized using the SILVER SEQUENCE TM DNA Sequencing System (Promega).

Statistical methods

The data were extracted as a table and marked as either present (1) or absent (0). Monomorphic bands (bands present in all individuals of a species) were discriminated within each species and across the entire data set. The binary matrix was analyzed with the program NTSYS-PC version 2.02 (Rohlf 1993). The unweighted pair-group arithmetic mean method (UPGMA) cluster analysis was performed on the simple matching (SM) association coefficient (Sneath & Sokal 1973), and the same program generated the phenogram showing similarity relations. The distortion produced during the grouping analysis was calculated using the correlation cophenetic coefficients (r) (Sokal & Rohlf 1962) using the NTSYSPC version 2.02 (Rohlf 1993) program. A cophenetic correlation (r) of 0.8 was considered a good fit. A three-dimensional graphic was obtained with the principal coordinated ordination method (Gower 1966).

The percentage of polymorphic loci (P = (number of polymorphic loci/ number of loci analyzed) × 100) for each primer combination was calculated.

Results

The analysis of the six primers that produced clear and reproducible bands by AFLP amplification yielded a total of 926 bands that appeared consistently in all experiments among the amplified fragments of the 31 isolates. Although the amplified bands ranged from 50 to 550 bp, we analyzed only bands from 90 to 330 bp.

Table 3 describes the total number of AFLP (polymorphic + monomorphic) bands and the percentage of polymorphic loci obtained for each selective primer combination in the three assayed fungal species. All primers detected polymorphic bands within species and a high percentage of polymorphic loci

Table 3. Total number of AFLP (polymorphic + monomorphic) bands detected for each selective primer combination per *Coprotus* species assayed.

Primer combination	Species	Total N° of bands	Polymorphic bands	Monomorphic bands	P%
M+T/E+ACG	*C. lacteus*	50	16	34	32
	C. niveus	27	7	20	26
	C. sexdecimsporus	72	60	12	83
M+C/E+AAG	*C. lacteus*	101	28	73	28
	C. niveus	108	10	98	9
	C. sexdecimsporus	163	141	22	86
M+A/E+AAG	*C. lacteus*	75	12	63	16
	C. niveus	90	38	52	42
	C. sexdecimsporus	152	135	17	89
M+C/E+ACG	*C. lacteus*	77	23	54	30
	C. niveus	129	75	54	58
	C. sexdecimsporus	156	144	12	92
M+G/E+ACG	*C. lacteus*	79	26	53	33
	C. niveus	80	36	44	45
	C. sexdecimsporus	100	74	26	74
M+A/E+ACG	*C. lacteus*	74	24	50	32
	C. niveus	70	14	56	20
	C. sexdecimsporus	143	130	13	90

were obtained for *C. sexdecimsporus* and a reasonable percentage for *C. niveus* and for *C. lacteus.*

Genetic variability within species, measured in terms of percentage of polymorphic loci was variable: P = 87 % for *C. sexdecimsporus,* P= 36 % for *C. niveus* and, P= 28 % for *C. lacteus.*

The primer group M+C/E+ACG was more effective in detecting polymorphisms (Table 3).

Degree of similarity and cluster analysis

The phenogram obtained using the UPGMA method was constructed based on Simple Matching (SM) coefficients. Little distortion occurred while constructing this phenogram, as implied by the value of the correlation cophenetic coefficient (r=0.991). There was no direct correlation between molecular genotype and geographic origin.

The UPGMA phenogram (Fig. 1) grouped all strains in three defined clusters that correspond to the three *Coprotus* species.

The degree of similarity among species was low. Between *C. lacteus* and *C. sexdecimsporus*, the coefficient of similarity was S = 0.5. The group formed

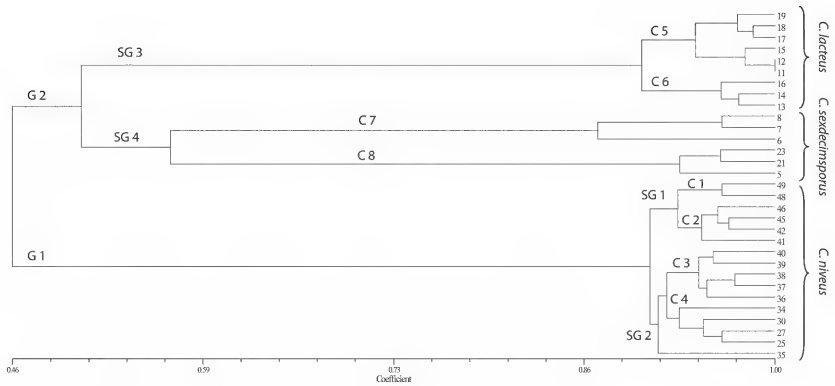

FIGURE 1. UPGMA phenogram showing relationships among *Coprotus lacteus, C. sexdecimsporus,* and *C. niveus* isolates based on the simple matching (SM) association coefficient estimated from AFLP loci.

by the isolates identified as *C. niveus* clustered with the other two species with a coefficient of similarity of S = 0.46.

The main group (FIG. 1, group G1) comprised two subgroups (SG1 and SG2) formed by all isolates of *C. niveus*. Subgroup SG1 is formed by two sets (C1 and C2) and SG2 subgroup comprised C3 and C4 sets and one isolate separated from the rest. A second group (FIG. 1, G2) included two subgroups (SG3 and SG4). Subgroup SG3 is formed by two sets (C5 and C6) that included all isolates of *C. lacteus*. The other subgroup (SG4) comprised C7 and C8 sets, formed by all isolates of *C. sexdecimsporus.*

Intra-specific variability detected for *C. lacteus* and *C. niveus* was low. This fact is reflected by the association coefficient: SM = 0.91 among isolates of *C. lacteus* and SM = 0.92 among *C. niveus* isolates. Only two pairs of isolates presented 100% of similarity (association coefficient 1.0), and they belonged to *C. lacteus*. *Coprotus sexdecimsporus* showed a higher degree of variability than the other species, with SM = 0.54 among all isolates of this species.

The ordination of isolates through the principal coordinated method allowed us to recognize three groups (FIG. 2, groups 1–3) in three-dimensional dispersion revealing nearly the same relations between isolates as the phenogram (FIG. 1). The first group included all isolates of *C. lacteus*, very closely attached in the three axes. The second set showed *C. sexdecimsporus* isolates differentiated in axis 1 but very closely in the other two axes. The third comprised isolates of *C. niveus* closely together in the three axes, thus revealing a high degree of similarity.

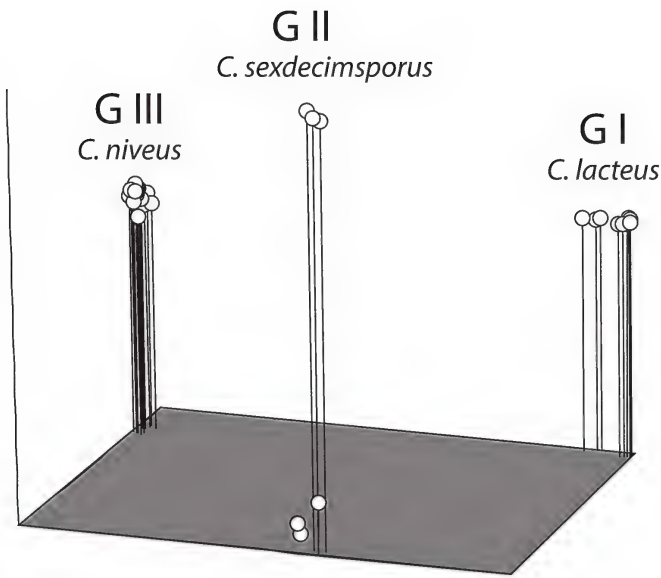

FIGURE 2. Three-dimensional graphic of *Coprotus* species obtained with the Principal Coordinates ordination technique.

Discussion

In the present study the high number of AFLP bands obtained with the six primers assayed allowed us to detect intra-specific variability.

Previous biochemical and RAPD studies detected a high intra-specific homogeneity (Suárez et al. 2006, Ramos et al. 2008), which could be related to the fact that *Coprotus* is homothallic and produces clonal offspring (Rayner 1994). In the present study the genetic variability within species, in terms of percentage of polymorphic loci, was two to three times higher than those obtained by RAPD. AFLP fingerprinting is more effective for detecting genetic diversity in species with high intra-specific homogeneity (e.g., *Coprotus*), because it generated a very large number of polymorphic loci.

Genetic diversity could be a potential indicator of the relative abundance of sexual and asexual reproduction (Chen & McDonald 1996). Furthermore other alternatives for genetic interaction among fungi besides sexual recombination are heterokaryosis and mitotic recombination (Esser & Kuenen 1967). In this

sense, natural selection could lead to genetic divergence and therefore increase genetic variability levels in the homothallic fungi as *Coprotus* (non-out crossing populations). The AFLP technique allowed us to detect such intraspecific variability.

In the phenograms generated by AFLP and RAPD markers, all strains of the same species group into three defined clusters. Besides, both phenograms showed very similar phenetic relationships among species.

The phenogram obtained from AFLP datasets showed a higher association between *C. lacteus* and *C. sexdecimsporus*. This result is consistent with that obtained by means RAPD markers. This agreement might be related to the nature of these markers since AFLP and RAPD are usually considered neutral markers. Sampling and genotyping the progeny within sexual structures in natural populations should allow determining whether haploid self-mating system actually occurs in nature (Billiard et al. 2012).

The AFLP technique could become a powerful tool for researching genera with homothallic mating systems such as *Coprotus*, in which a high intraspecific homogeneity does not allow detection of variability using other PCR-based markers.

Acknowledgements

This study was supported by a grant from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and University of Buenos Aires, Argentina. The authors thank Dr. Lina Bettucci (Universidad de la República, Montevideo, Uruguay) and Dr. Alejandra Fazio (Universidade de São Paulo, Brasil) for expert presubmission review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.275

Volume 130, pp. 275–278 *January–March 2015*

Verticicladus hainanensis, a new aquatic hyphomycete

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Abstract —*Verticicladus hainanensis*, isolated from decaying leaves in Jianfengling Nature Reserve, Hainan Province, China, is described and illustrated. It is characterized by fusoid, 5–8-septate, slightly grey to dark fuliginous conidia.

Key words —fungal diversity, morphology, freshwater fungi, taxonomy

Introduction

The fungal genus *Verticicladus* is characterized by rhexolytic conidial liberation from conidiogenous cells verticillately fasciculated on short cylindrical conidiophores (Matsushima 1993). Two species have been described, *V. amazonensis* Matsush. and *V. subiculifer* Matsush. (Matsushima 1993, 1996).

China has an enormous fungal diversity occurring on dead branches, rotten submerged wood, and leaves. Many anamorphic fungi collected in southern China have been published recently (Zhang et al. 2009a,b,c, 2011, 2012; Ma et al. 2010, 2011a,b, 2012a,b; Wang et al. 2011; Ren et al. 2011, 2012). A species isolated from decaying leaves in a small river from Jianfengling Nature Reserve, Hainan Province, China had the characteristics of the genus *Verticicladus* but differed from the two described species. Here, the new species is described and illustrated in detail.

Materials & methods

The culture was isolated from dicotyledonous leaves submerged in a river in Hainan. The rotten leaves were scissored to several 2–4 × 2–4 cm sized fragments and then spread on to the surface of CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin,

[§]Min Qiao & Ming-Tian Guo contributed equally to this work

30 mg ampicillin, 1000 ml distilled water) medium for ten days; single conidia were isolated using sterilized needles while viewing with a BX51 microscope, and cultivated on CMA in Petri plates. Morphological observations were made from CMA after incubation at 28°C for one week; pure cultures and a permanent slide were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF; formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

Taxonomy

Verticicladus hainanensis M.T. Guo & Z.F. Yu, **sp. nov.** PLATE 1

MYCOBANK MB 809830

Differs from *Verticicladus amazonensis* by its shorter, fusiform conidia and from *V. subiculifer* by its attenuate conidia.

TYPE: PR China, Hainan Province, Jianfengling Nature Reserve, Chinese Academy of Science, 18°44′N 108°49′E, elev. 682 m, in a river on submerged leaves of an unidentified dicotyledonous plant, 23 June 2011, G. Z. Yang (**holotype**, YMF1.04024 [dried agar culture]; permanent slide, YMF1.040241; ex-type cultures, YMF1.040242).

ETYMOLOGY: *hainanensis* refers to the province in which the species was found.

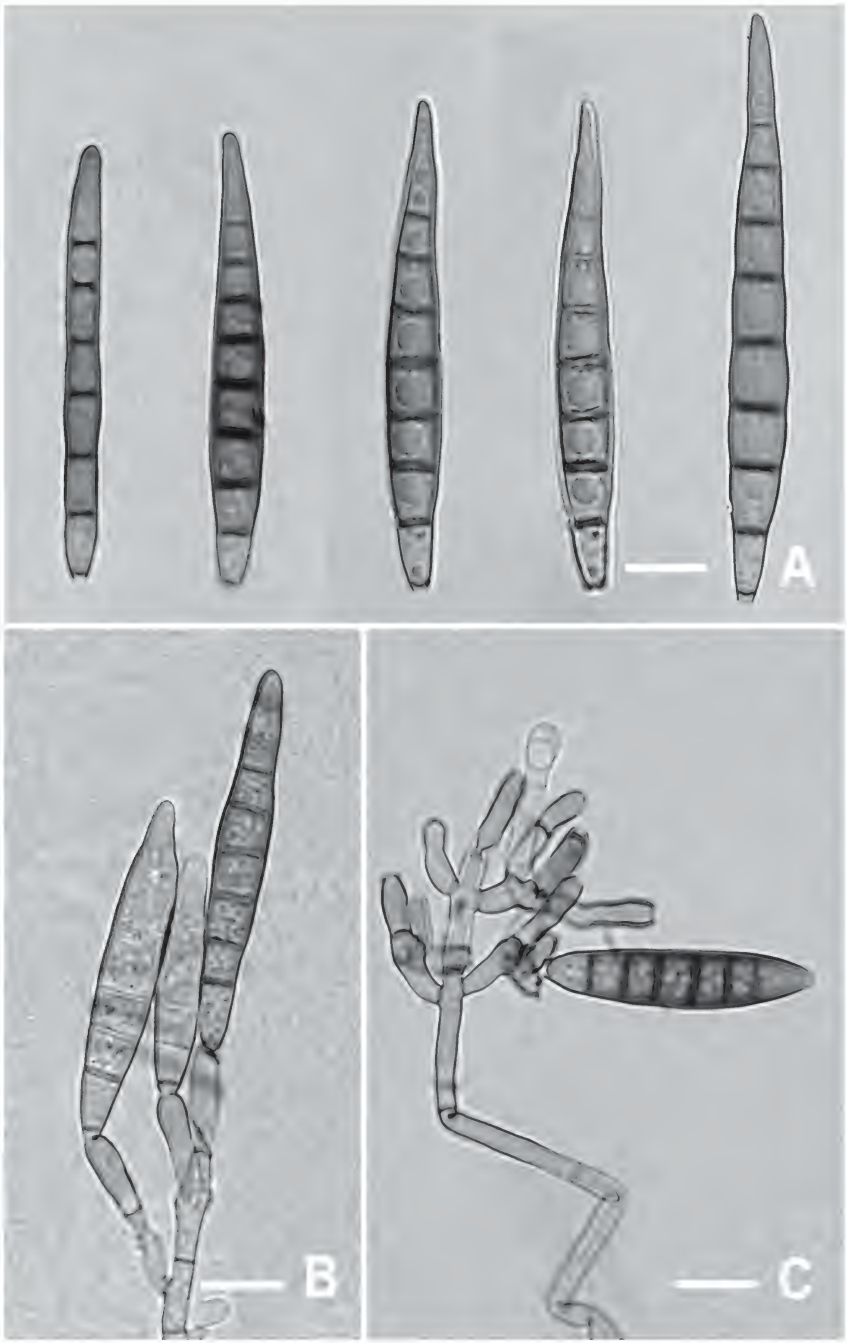
COLONIES growing moderately on CMA, attaining about 15 mm diam. after 7 days cultivation on CMA at 28°C. Colony effuse, dark olive to fuliginous, mycelium partly superficial, partly immersed in substratum, composed of branched, septate, smooth hyphae. Vegetative hyphae 1.9–3.8 µm wide, often 2.5–3.5 µm wide, hyaline to fuliginous. Conidiophores arising laterally on hyaline vegetative hyphae, cylindrical with the apical part rounder, 6.75–9.65 × 2.35–3.5 µm, hyaline to slightly grey. Conidiogenous cells cylindrical, with obvious truncations at the apex, 5.9–14.4 × 2.1–3.7 µm, slightly grey, attaching directly on the terminal part of hyphae, solitary or on the conidiophores in aggregates of 3 or more. Conidia fusoid, conspicuously attenuated from the mid-part to the apex, slightly grey to dark fuliginous, smooth at surface, 37.55–65.15 × 4.2–7.6 µm, 5–8-septate, often 7-septate, thick-walled, with rhexolytic liberation from conidiogenous cells.

Discussion

Verticicladus hainanensis fits well within the genus *Verticicladus* based on its conidiogenous cells that are verticillately fasciculate on short cylindrical conidiophores and the multiseptate conidia with rhexolytic liberation from conidiogenous cells.

Verticicladus hainanensis is similar to *V. amazonensis*, which differs by its longer, cylindrical conidia (45–95 µm; Matsushima 1993); and *V. subiculifer*

PLATE 1. *Verticicladus hainanensis* (holotype, YMF1.04024). A. Conidia; B. Conidiogenous cells and conidia, C. Conidiophores and a conidium. Scale bars: A–C = 10 µm.


A
B
C

differs from *V. hainensis* by the different shape of its similar sized, cylindro-fusiform conidia lacking any obvious apical attenuation (Matsushima 1996).

Acknowledgements

This work was jointly financed by National Basic Research Program of China ('973'Program: 2013CB127506) and National Natural Science Foundation Program of PR China (31160008, 31260007, 31360130), Grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI020). We are very grateful to Prof. X.G. Zhang and Dr. R.F. Castañeda-Ruiz for critically reviewing the manuscript and providing helpful suggestions to improve this paper.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.279

Volume 130, pp. 279–287 *January–March 2015*

Geographic distribution of *Sarcoporia polyspora* and *S. longitubulata* sp. nov.

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Abstract — DNA study of *Sarcoporia polyspora* (= *Parmastomyces transmutans*) revealed only negligible sequence differences between conifer-dwelling specimens with cartilaginous layer in the context from USA, Brazil, Europe, and Far East Asia, but a very different sequence from three resupinate and hardwood-bound collections without such a layer and with slightly narrower and pale brown spores from USA and Madeira Islands. This fungus, found also among historical USA collections of *S. polyspora* in the BPI herbarium, is described here as *Sarcoporia longitubulata*. The phylogenetic position of *Sarcoporia* is discussed.

Key words — *Basidiomycota,* brown rot fungi, molecular taxonomy, *Parmastomyces kravtzevianus*

Introduction

Sarcoporia polyspora P. Karst. is a very distinct, brown-rot polypore with soft, resupinate to effused-reflexed basidiomes that are white to crème at first but turn reddish-brown after bruising or drying. It can be easily recognized by its ellipsoid and thick-walled, dextrinoid spores, which are quite unique in polypores. The species is rare in Europe but rather common in North America (on *Tsuga* spp. and *Pinus* spp.) and in Asia (Vlasák & Kout 2010, Dai 2012). The fungus was described by Karsten (1894) but Karsten's name was abandoned for years. Based on collections from Estonia and Siberia, it was independently described by Parmasto (1957) as *Tyromyces kravtzevianus* Bondartsev & Parmasto, and a few years later Kotlaba & Pouzar (1964) created the genus *Parmastomyces* for this unique polypore. In the USA, Overholts (1941) described this species (invalidly, without a Latin diagnosis) as "*Polyporus subcartilagineus.*" Later he described still another species, *Polyporus*

transmutans Overh., which he distinguished by the more distinctly pileate habit and its growth on *Prunus* (Overholts 1952). Ryvarden & Gilbertson (1984), who found the type of *P. transmutans* identical with "*P. subcartilagineus*" as well as with European specimens of *Parmastomyces kravtzevianus*, coined the name *Parmastomyces transmutans* (Overh.) Ryvarden & Gilb. for all collections with typical spores. Niemelä et al. (2005), however, studied Karsten's type material and re-established the oldest name, *Sarcoporia polyspora*, for this species.

Somewhat aberrant morphology of some recent collections from hardwood hosts and conifers led us to question the identity of Eurasian/American and of hardwood/conifer-inhabiting *Sarcoporia*. We have sampled European, American, and Asian collections from conifers and hardwoods and also studied the type of *Polyporus transmutans* from the BPI herbarium along with several other American BPI collections determined as *P. transmutans* to explore whether or not all these specimens represent the same species.

Materials & methods

Morphological study

The studied specimens are deposited in the US National Fungus collection, Beltsville, USA (BPI), in private herbarium of J. Vlasák (JV), and in mycological herbarium of Finnish Museum of Natural History, University of Helsinki, Finland (H). They were examined from slides prepared in cotton blue (CB) and Melzer's reagent (IKI) with a Leitz Diaplan microscope (×1250 magnification). Measurements were done in cotton blue using phase contrast illumination and oil immersion (with a subjective accuracy of 0.1 μm; Miettinen et al. 2006). For presenting the size range of basidiospores, 5% of the measurements were excluded from each end of the range and are given in parentheses. The following abbreviations are used: L′ = basidiospore length, L = mean basidiospore length (arithmetical average of all basidiospores), W′ = basidiospore width, W = mean basidiospore width (arithmetical average of all basidiospores), Q = L′/W′ ratio range, and n = the number of basidiospores measured from given number of specimens. KOH– indicates unchanged in 5% KOH and IKI– indicates unchanged in Melzer's reagent.

Molecular phylogeny

DNA isolation and nrDNA ITS region sequencing methods follow Vlasák & Kout (2011). The sequences were aligned with Clustal X and manually pruned. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013) using "all sites" and "uniform rates" options. Other options, such as "complete deletion" and "gamma distributed with invariant sites," and all their combinations provided virtually identical phylogeny.

Results

Phylogenetic analysis

Sarcoporia specimens collected on hardwoods and conifers in the USA, Madeira Island, Czech Republic, and European and Far East Russia were

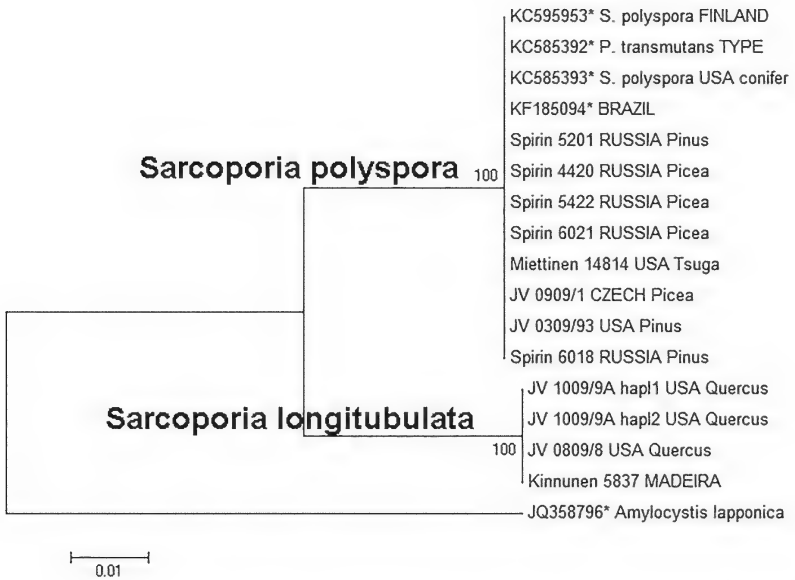

FIGURE 1: Phylogenetic relationships of 15 *Sarcoporia* specimens inferred from ITS rRNA sequences. *Amylocystis lapponica* was used as outgroup. Topology from maximum likelihood (ML) analysis. Support values along branches from ML bootstrap (1000 replicates). Branch lengths are drawn proportional to the number of substitutions per site. GenBank numbers with asterisk indicate sequences retrieved from GenBank; other GenBank numbers in Specimens examined.

sequenced and compared with *Sarcoporia* sequences in GenBank. A total of 14 ITS and 2 nucLSU sequences were newly generated for this study and deposited in GenBank. The ITS dataset comprising 16 sequences resulted in an alignment with 653 characters, of which 81 were variable and 30 parsimony informative. Extremely high sequence conservation could be demonstrated among all conifer-dwelling specimens from different continents, with Eurasian specimens differing from American in only one base insertion in ITS1 and some specimens showing two haplotypes, with and without this insertion (not shown in FIG. 1). On the other hand, most of hardwood-dwelling specimens showed a very different sequence with about 40 mutations in ITS region. Similarly large sequence differences were found with the nucLSU sequence dataset (not shown), using only two sequences from each species. The aberrant hardwood specimens were also characterized by very thick and soft resupinate basidiocarps with long tubes and thin subiculum without cartilaginous layer. They are described below as a new species, *S. longitubulata*.

Taxonomy

Sarcoporia longitubulata Vlasák & Spirin, **sp. nov.** Figs 2– 4

MycoBank MB 809624

Sarcoporiae polysporae *similis, sed tubulis longis et sporis coloratis.*

Type: USA, Pennsylvania: Phoenixville, Schuylkill Canal, Ravine Trail, log of *Quercus*, 2 Sep 2008, J. Vlasák JV 0809/8 (**Holotype**, BPI 892956; **isotypes**, JV, H; GenBank, KM207860, KM207863).

Etymology: *longitubulata* (Lat.), referring to the very long tubes found in most of the collected specimens.

Basidiomes annual, resupinate, pulvinaceous, up to 20 cm long, 15 cm wide, and 2 cm thick, strikingly soft to downy, drying brittle; margin whitish to crème, about 1 mm broad, soft and cottony, later indistinct; pore surface at first crème with reddish tints, distinctly reddish-brown after bruising, brown to blackish-brown on drying, the pores circular, with thin dissepiments, 3–5 per mm; context 1–3 mm thick, soft and cottony, persistently crème colored; tube layer very thick, up to 20 mm, extremely brittle and shattering easily when dry, crème at first but deep brown when dry and contrasting with crème subiculum. Spore print brown.

Hyphal structure monomitic; hyphae with clamp connections, KOH–, IKI–. Context. Hyphae arranged in subparallel bundles, mostly thin-walled, 4–10 µm in diam., some hyphae with thick-walls and refractive, hyaline or pale

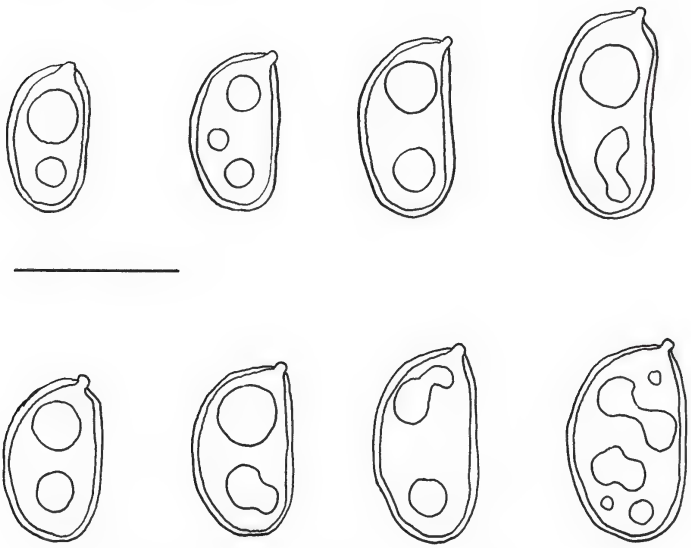

Figure 2: *Sarcoporia* basidiospores: above – *S. longitubulata* (holotype); below – *S. polyspora* (lectotype). Scale bar = 5 µm.

FIGURE 3: *Sarcoporia longitubulata* (JV 0809/8, holotype), underside of oak log, photo in situ. Spore deposits below left.

brown, with cyanophilous content, strongly inflated at septa, 7–18(–22) µm in diam., occasionally with double clamps. TUBES. Hyphae subparallel, rather loosely arranged, thin-walled, hyaline, easily collapsing, some with cyanophilous content, (3.5–)4–5(–6) µm in diam. Cystidia absent. BASIDIOSPORES thick-walled, brownish, oblong-ellipsoid, ventral side slightly concave or rarely flat, dextrinoid, a few spores weakly or moderately cyanophilous, (4.3–)4.4–6.2 (–7.2) × (2.3–)2.5–3.3(–3.5) µm (n = 150/5), L = 5.29, W = 2.88, Q = 1.78–1.93.

DISTRIBUTION & ECOLOGY. On hardwood logs (predominantly *Quercus* spp.) in the US North-East (Michigan, New York, Pennsylvania, Tennessee) and Macaronesia (Madeira Is.); causing a brown rot.

ADDITIONAL SPECIMENS EXAMINED: **PORTUGAL. MADEIRA**: Madeira Island, 3 km W from Funchal, fallen angiosperm logs, 19 Jan 2011, Kinnunen 5837 (H, GenBank KM207865), 5845, 5848 (H). **USA. PENNSYLVANIA**: Gettysburg, Big Round Top, *Quercus*, 5 Sep 2010, Vlasák JV 1009/9A (JV, H, GenBank KM207861, KM207862, KM207864). **MICHIGAN**: Ann Arbor, hardwood, Jul 1958, Wehmeyer (BPI 223824). **NEW YORK**: Rye, *Quercus*, 30 Oct 1980, Stein (BPI 222859). **TENNESSEE**: Great Smoky Mt., New Found Gap, *Fagus*, 9 Sep 2005, Vlasák JV 0509/103 (JV, H).

COMMENTS. Macroscopically, *S. longitubulata* is very distinct, so that we felt certain from first glance that it must be a different species (FIGS 3, 4). The brown spore deposits around the basidiocarp, the very long and soft tubes, and the relatively thin subiculum lacking a cartilaginous layer are its most striking morphological features. *Sarcoporia polyspora* produces white spore deposits and more or less pileate basidiocarps with short tubes and a thick subiculum containing a distinct cartilaginous layer. We noted no odor of fresh

FIGURE 4: *Sarcoporia longitubulata* (JV 0809/8, holotype), underside of oak log, photo in situ.

basidiocarps, but on drying, *S. longitubulata* smelled so repugnantly that it was impossible to keep in the same room for more than one week, although it was not contaminated with yeasts. The primary microscopic difference of *S. longitubulata* is the pale brown coloration of the basidiospores, visible in cotton blue even with low resolution; only a few spores are more or less stained (slightly or moderately cyanophilous). In contrast, *S. polyspora* basidiospores are exceptionally strongly cyanophilous (uniformly bright blue), so that their natural color is totally undetectable in cotton blue. Moreover, the *S. longitubulata* spores are a bit narrower than in *S. polyspora* (TABLE 1) and less variable in shape and size, being uniformly oblong-ellipsoid and slightly concave on their ventral side (FIG. 2). The basidiospores of this type occur in *S. polyspora*, too, but only as a rare variation.

Discussion

We have shown that *S. polyspora* is widely distributed from America to Europe and Far East Asia. The slight morphological differences we observed—e.g., longer spores and more pileate habit in USA collections vs. shorter spores in Far East Asian collections—have no support in DNA sequence differences and so should be regarded as regional morphological variation. A tropical distribution remains uncertain, as the sequenced specimens from Brazil were collected from *Pinus* logs probably imported from the USA (Baldoni et al. 2015).

All studied collections were from conifers except for BPI 844703, the type of *Polyporus transmutans* (Overholts 1952), collected on *Prunus serotina* Ehrh. in northwestern USA. This specimen displays strikingly large and tough pilei with

Table 1. Spore measurements of two *Sarcoporia* species. (Limit and mean values for each species shown in bold.)

Species / specimen	L′	L	W′	W	Q	n
S. polyspora	**(4.2)4.7–7.2(7.3)**	**5.53**	**(2.5)2.6–3.8(4.0)**	**3.10**	**1.79**	**270**
Miettinen 14814	(5.0)5.1–6.3(6.7)	5.64	(2.7)2.8–3.6(4.2)	3.14	1.81	30
Spirin 4420	(4.9)5.0–6.3(6.7)	5.51	(2.7)2.8–3.2(3.3)	2.97	1.86	30
Spirin 5201	(4.2)4.7–5.7(5.8)	5.05	(2.6)2.7–3.3	3.02	1.68	30
Spirin 5422	(4.7)4.8–5.7(7.1)	5.22	(2.5)2.6–3.2(3.3)	2.93	1.79	30
Spirin 6021	(4.8)5.2–6.8(7.3)	5.70	(2.6)2.7–3.6(3.7)	3.07	1.86	30
Spirin 6018	(4.8)4.9–6.1(6.2)	5.30	(2.7)2.8–3.4(3.7)	3.11	1.71	30
Vlasák 0908/15	(4.8)5.0–6.2(6.4)	5.56	(2.7)2.8–3.7(3.9)	3.17	1.76	30
Vlasák 0309/93	(5.2)5.3–7.2(7.3)	6.27	(2.8)2.9–3.8(4.0)	3.19	1.97	30
S. longitubulata	**(4.3)4.4–6.2(7.2)**	**5.29**	**(2.3)2.5–3.3(3.5)**	**2.88**	**1.84**	**150**
Vlasák 0809/8	(4.3)4.4–5.8(6.0)	5.03	(2.6)2.7–3.1(3.3)	2.82	1.79	30
Vlasák 1009/9a	(4.6)4.8–6.2(6.3)	5.42	(2.3)2.5–3.2(3.3)	2.82	1.93	30
Vlasák 0509/103	(4.4)4.8–5.8(7.2)	5.30	(2.7)2.8–3.3(3.5)	2.97	1.78	30
BPI 222859	(4.8)4.9–6.2(6.7)	5.39	(2.6)2.7–3.2(3.3)	2.90	1.86	30
BPI 223824	(4.8)4.9–6.0	5.32	(2.5)2.6–3.3(3.4)	2.91	1.83	30

short tubes and thick context in which no distinct gelatinous layer is present but scattered gelatinous streaks are present. This suggests just a very large specimen of *S. polyspora* and our examination revealed no distinct microscopic differences from other *S. polyspora* specimens. Moreover, the ITS sequence of the culture derived from this specimen available in GenBank (KC585392; Ortiz-Santana et al. 2013) is identical with other *S. polyspora* sequences.

When we sought for a possible existing name for *S. longitubulata*, *Tyromyces mollissimus* Maire, described from North Africa (Maire 1945) and presently regarded as a synonym of *S. polyspora,* seemed a reasonable candidate. Unfortunately, the type material is now unavailable (Pouzar 1984, Kotiranta 1998). Nevertheless, the protologue of *T. mollissimus* is very clear and leaves no doubts about its identity: it was described as growing on *Pinus halepensis* Mill. and having pileate or effused-reflexed, very soft basidiocarps with thick contextual layer (see fig. 7b, Maire 1945: 37), and a white spore print. All these features fit well with the current concept of *S. polyspora* and certainly rule out our new species. The recently described *Sarcoporia neotropica* from Costa Rica (Ryvarden 2013) has imbricate soft basidiocarps and hyaline basidiospores, characters that preclude its identity with *S. longitubulata*.

Because of similar spores and some of tissue features, Ryvarden & Melo (2014) speculated about *Sarcoporia*'s position in the *Coniophoraceae.* Molecular phylogeny refutes this hypothesis, however. The sequences of typical

coniophoraceous species such as *Coniophora puteana* (Schumach.) P. Karst. and *Serpula lacrymans* (Wulfen) J. Schröt. differ greatly from *Sarcoporia*, which has already been recognized as a member of "antrodia clade" within the *Polyporaceae,* most closely related to *Amylocystis* and *Auriporia* (Ortiz-Santana et al. 2013). *Amylocystis lapponica* (Romell) Bondartsev & Singer, is in fact quite similar, being soft and turning reddish brown after bruising and drying, causing a brown rot, and with some microstructures staining in Melzer's reagent. *Bondarcevomyces taxi* (Bondartsev) Parmasto, which was once referred to *Parmastomyces* (= *Sarcoporia* in current sense) by Dai & Niemelä (1995), belongs to the *Boletales* (Binder & Hibbett 2006). It does share some characters with *S. longitubulata* (brownish spores, strong pungent odor of drying basidiocarps) although they indicate only superficial similarity.

Additional material examined: ***Sarcoporia polyspora.*** **CZECH REPUBLIC.** Hluboká, Karluv Hradek, *Picea abies*, 2 Sep 2009 Vlasák JV 0909/1 (JV, GenBank KM207858, KM207859), 11 Aug 2009, Vlasák JV 0908/15 (JV, PRM 915664, H). **FINLAND. Uusimaa:** Inkoo, Fagervik, Sept 1893, Hisinger (H) (lectotype of *S. polyspora*; Lowe 1956). **RUSSIA. Khabarovsk Reg.:** Khabarovsk Dist., Ulika, *Pinus koraiensis*, 13 Aug 2012, Spirin 5201 (H, GenBank KM207856, KM207857), Malyi Kukachan, *Picea ajanensis*, 19 Aug 2012, Spirin 5422 (H, GenBank KM207852); Solnechnyi Dist., Evoron, *Picea obovata*, 27 Aug 2011, Spirin 4420 (H, GenBank KM207853). **Nizhny Novgorod Reg.:** Lukoyanov Dist., Panzelka, *Pinus sylvestris*, 8 Aug 2013, Spirin 6018 (H, GenBank KM207851), *Picea abies*, 8 Aug 2013, Spirin 6021 (H, GenBank KM207854, KM207855). **USA. Massachusetts:** Holden, Quinapoxet, *Tsuga canadensis*, 26 Sep 2011, Miettinen 14814.1 (H, GenBank KM207850). **Pennsylvania:** Wilkes-Barre, Ricketts Glen St. Park, *Pinus*, 11 Sep 2003, Vlasák JV 0309/93 (JV, H, GenBank KM207849); Haycock, Nockamixon St. Park, *Pinus strobus*, 7 Aug 2004, Vlasák Jr. JV 0408/4J (JV); McKean Co., *Prunus serotina*, 10 Sep 1940, Campbell & Davidson 22971 (BPI 844703, holotype of *Polyporus transmutans*, GenBank KC585392). **New York:** Adirondack Park, Keene Valley, Johns Brook Trail, conifer, 23 Sep 2005, Vlasák Jr. JV 0509/193J (JV).

Bondarcevomyces taxi. **RUSSIA. Khabarovsk Reg.:** Khabarovsk Dist., Bol'shoi Khekhtsir Nat Res., *Picea ajanensis*, 5 Sep 2013, Spirin 6674 (H, JV); Komsomol'sk Dist., Boktor, *Larix gmelinii*, 18 Aug 2013, Spirin 6141 (H, JV).

Acknowledgments

The authors thank the BPI herbarium staff for the kind loan of *Sarcoporia* specimens, Dr. Otto Miettinen for sequencing the Kinnunen 5837 specimen, and Prof. Teuvo Ahti for revising the Latin diagnosis. Valuable discussions with Jiri Kout, G. Coelho, and Y.-C. Dai and their contributions as presubmission reviewers are acknowledged. The research of J. Vlasák was funded by institutional support RVO: 60077344.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.289

Volume 130, pp. 289–293 *January–March 2015*

Coccomyces neolitseae sp. nov. from Zhangjiajie, China

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Abstract — A fungus found on leaves of *Neolitsea levinei* from the Zhangjiajie National Forest Park in Hunan Province, China, is described as *Coccomyces neolitseae*. A description, illustration, and comments are given for this taxon. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — *Rhytismataceae*, *Rhytismatales*, morphological characteristics, taxonomy, *Lauraceae*

Introduction

Coccomyces De Not. is now a large genus in the *Rhytismataceae* (Kirk et al. 2008). The ascomal external shape, size, and type of opening, the ascal shape and size, and the shape of paraphyses at the apex are regarded as the most important taxonomic characters of *Coccomyces* (Johnston 1986, Lin 1998). Index Fungorum (2015) lists 196 epithets for the genus, of which Species Fungorum (2015) accepts 106 species in *Coccomyces*. They usually occur on leaves, herbaceous stems, bark, and wood of vascular plants, especially *Ericaceae*, *Fagaceae*, and *Lauraceae* (Sherwood 1980). Some species, such as *C. guizhouensis* Y.R. Lin & B.F. Hu, *C. ledi* Rehm, *C. strobi* J. Reid & Cain, and *C. vilis* Syd. et al., may cause economically significant plant diseases (Lin et al. 1994, Rehm 1913, Reid & Cain 1961, Cannon & Minter 1984).

Recently, another new *Coccomyces* species was found on fallen leaves of *Neolitsea levinei* in the Zhangjiajie National Forest Park, Hunan Province, which we describe below.

Materials & methods

The study was based on collections made in 2010 from Hunan, China. Specimens were rehydrated in water for 15 min and ascomata and conidiomata were sliced into 10–

15 µm thick sections using a freezing microtome. The structure of the fruiting bodies was observed in 0.1% (w/v) cotton blue in phenol glycerin. The color of the internal structures of the fruiting bodies was observed in water. Measurements and drawings of asci, ascospores, and paraphyses were made from squash mounts in 5% KOH solution.

Taxonomy

Coccomyces neolitseae Qing Li & Y.R. Lin, **sp. nov.** FIGS 1–6

MycoBank MB 809422

Differs from *Coccomyces delta* by the lack of lip cells, the existence of an excipulum, a thicker basal stroma, paraphyses that are not enlarged at the apex, shorter and thinner ascospores lacking an obvious gelatinous sheath, and the ascomata associating with conidiomata.

Type: China, Hunan, Zhangjiajie National Forest Park, Jinbianxi, on fallen leaves of *Neolitsea levinei* Merr. (*Lauraceae*), 17 August 2010, X.M. Gao & Y.R. Lin 2546 (**Holotype**, AAUF 68654).

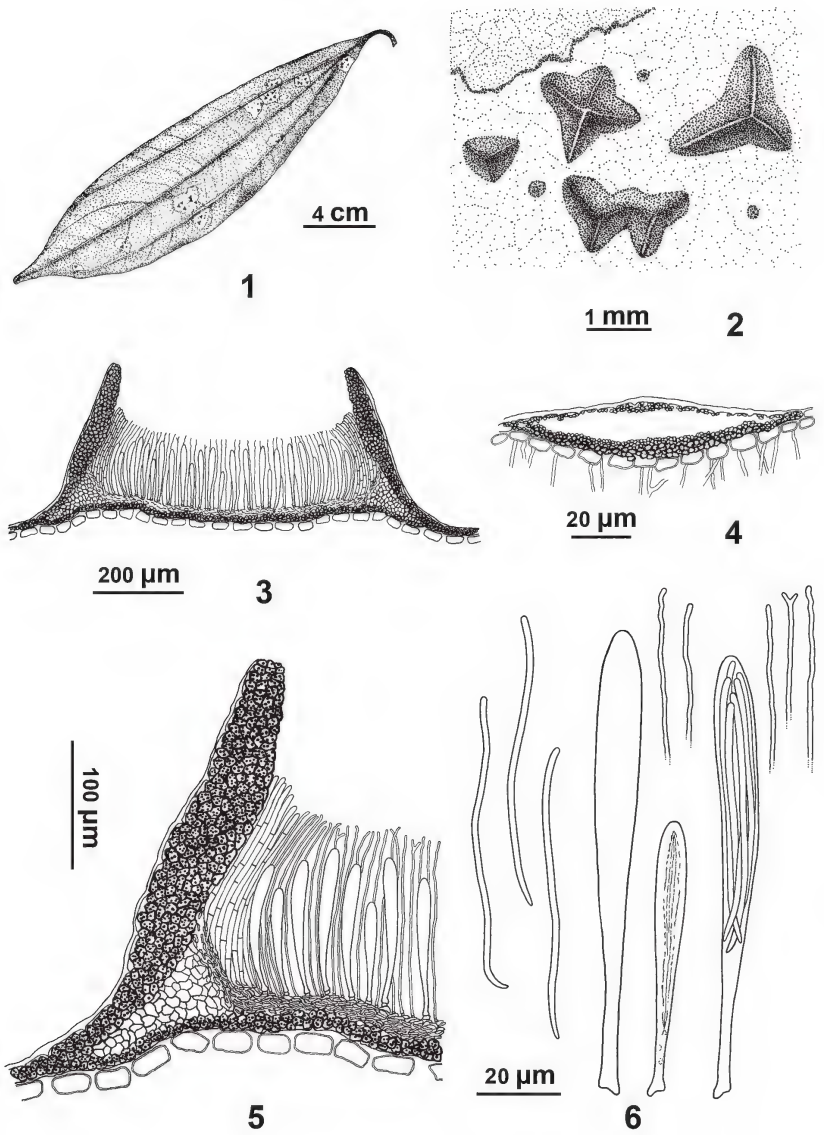
Etymology: The specific epithet refers to the host genus.

Colonies on both sides of leaves, forming irregular, yellowish-white spots 6–20 mm diam.

Zone lines frequent, dark-brown or black, thin, entirely or partly surrounding the bleached areas.

Conidiomata on both sides of leaves, predominantly on the lower side, scattered to clustered, sometimes with several coalescing. In surface view, conidiomata 110–215(–250) µm diam., subcircular, brown to black-brown, with a marked perimeter line or clearly defined edge, somewhat raising the substratum surface, discharging spores through an apical ostiole. In vertical transverse section, conidiomata intraepidermal, more or less lens-shaped. Upper wall 3.5–6.5 µm thick, dark brown, composed of tiny, thin-walled angular cells 1–1.5 µm diam. Basal wall 2.5–6 µm thick, comprised of (1–)2–3 layers of thick-walled angular cells 2–3 µm diam. Subconidiogenous layer 2.5–4 µm thick, consisting of nearly colorless, thin-walled angular cells. Conidiogenous cells and Conidia not present in material available.

Ascomata in similar positions to conidiomata on the substratum, scattered, occasionally two coalescing in the bleached spots. In surface view, ascomata 800–1200(–1600) µm diam., triangular, occasionally quadrilateral, black, with a distinct outline, moderately raising the substratum surface, with an obvious preformed dehiscence mechanism, opening by 3–4 radial splits that extend nearly to the ascomatal edge to expose the light yellow-brown hymenium. Lips absent. In median vertical transverse section, ascomata intraepidermal. Covering stroma 45–55 µm thick in the middle of the laterals, becoming thinner towards the top and the edge, connecting to the basal stroma, composed of black-brown textura angularis with thick-walled cells 6–8



Figs 1–6. *Coccomyces neolitseae* (holotype, AAUF 68654) on *Neolitsea levinei*. 1. Habit on a leaf. 2. Detail of conidiomata, ascomata, and a zone line. 3. Ascoma in median vertical transverse section. 4. Conidioma in vertical transverse section. 5. Portion of ascoma in median transverse vertical section. 6. Paraphyses, asci, and ascospores.

μm diam. Basal stroma 10–18 μm thick, consisting of textura angularis with 2–3 layers of black-brown, thick-walled cells. Internal matrix of stroma existing only between covering stroma and basal stroma, composed of colorless, thin-walled angular cells 7–12 μm diam., Excipulum well-developed, 25–35 μm wide, arising from the edge of the subhymenium, comprised of rows of hyaline, thin-walled, septate hyphae. Subhymenium 12–20 μm thick, composed of colorless textura porrecta-intricata. Paraphyses hyaline, aseptate, 120–150 × 0.8–1.4 μm, filiform, sinuate, not swollen, occasionally branched at the apex, not immersed in a gel. Asci ripening sequentially, 105–120 × 8–11 μm, clavate, long-stalked, thin-walled, apex rounded to obtuse, without circumapical thickening, J–, 8-spored. Ascospores arranged in a fascicle, 64–75 × 1.5–1.7 μm, filiform, hyaline, aseptate, gradually tapered towards the acute base, not obviously sheathed.

Host species, habitat, & distribution: Producing conidiomata and ascomata on fallen leaves of *Neolitsea levinei*; known only from the type locality, Hunan Province, China.

Comments — *Coccomyces delta* (Kunze) Sacc. is closely related to *C. neolitseae* in ascomal appearance and in the way it is embedded, as well as the characteristics of the zone lines. However, *C. delta* differs in the lack of an association of its ascomata with conidiomata, the absence of an excipulum, the presence of a fringe of periphysis-like lip cells, a much thinner (5 μm thick) basal stroma, slightly enlarged paraphysial apices, short-stalked, longer (100–150 μm) asci, and wider and longer (80–100 × 2 μm) ascospores with a narrow mucous sheath (Sherwood 1980). *Coccomyces delta* has been observed on a wide range of woody plants, mostly in the Europe and Atlantic islands, but with some records from China (Sherwood 1980; Lin et al. 2012). In our Chinese materials, the paraphyses are septate and covered by a thin mucous coating, and the asci and ascospores are shorter than described by Sherwood (1980).

Coccomyces limitatus (Berk. & M.A. Curtis) Sacc. is easily distinguished from the new species by trilateral to pentagonal ascomata, a covering stroma that does not extend as far as the basal stroma, a well-developed internal matrix comprising loose hyphae and crystals, a wider (50 μm thick) excipulum arising from inner layers of upper wall, wider (2–3 μm wide) paraphyses with swollen upper apices, and dissimilar asci and ascospores. Its asci are 4–5.6 μm, sometimes with an apical thickening, and the ascospores are only 0.8–1 μm wide and do not taper to the base (Johnston 1986).

Coccomyces neolitseae resembles *Coccomyces huangshanensis* Y.R. Lin & Z.Z. Li in the nature of the internal matrix of stroma and the color of the exposed hymenium, but *C. huangshanensis* has a much thinner covering stroma (8–17

μm) covered by heavily carbonized tissue at the opening that thins gradually towards the ascomal edge, short and hyaline periphysoids, and ascospores sometimes with a gelatinous cap at the apex (Lin et al. 2000).

Acknowledgements

The authors are grateful to Dr J.E. Taylor (Royal Botanic Garden Edinburgh, UK) and Dr M. Ye (Hefei University of Technology, China) for serving as pre-submission reviewers and to the Dr L.H. Wang for the identification of host plant. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31170019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.295

Volume 130, pp. 295-297 *January–March 2015*

Datronia ustulatiligna sp. nov. (*Agaricomycetes*) from India

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Abstract — A new poroid species, *Datronia ustulatiligna,* is described on partly burned gymnospermous wood from the state of Himachal Pradesh in India.

Key words — *Basidiomycota*, Rampur, Habban

While conducting fungal forays in the Narkanda, Rampur, Rajgarh, and Habban areas of the Shimla and Sirmaur districts in Himachal Pradesh (India), Harpreet and Dhingra made some collections of an unknown poroid fungus associated with partly burned trees of *Pinus roxburghii* and *Cedrus deodara.* After comparing macroscopic and microscopic features with published descriptions (Bakshi 1971, Ryvarden & Gilbertson 1993, Núñez & Ryvarden 2001, Bernicchia 2004, Sharma 2012), we identified the polypore as a new species of *Datronia* close to *D. scutellata*. It is distinguished by a combination of its association with partly burned gymnospermous wood, larger pores (2–4 per mm), and long narrowly clavate basidia.

Datronia ustulatiligna Har. Kaur, G. Kaur & Dhingra, **sp. nov.** Plate 1

MycoBank MB 810285

Differs from *Datronia scutellata* in having thicker basidiocarps, larger pores, and long, narrowly clavate basidia.

Type: India, Himachal Pradesh, Shimla, 8 km from Narkanda towards Rampur, on a partly burned tree of *Pinus roxburghii* Sarg., 2 August 2010, Harpreet Kaur 5609 (PUN, **holotype**).

Etymology: The epithet refers to the substrate, partly burned wood.

Basidiocarps annual to biennial, pileate, effused-reflexed to sessile, solitary to imbricate; up to 4 cm wide, 2.2 cm in radius, 2.2 cm thick near base, applanate to ungulate to dimidiate; abhymenial surface scrupose to somewhat hirsute, concentrically sulcate and zonate, often covered with mosses, brown

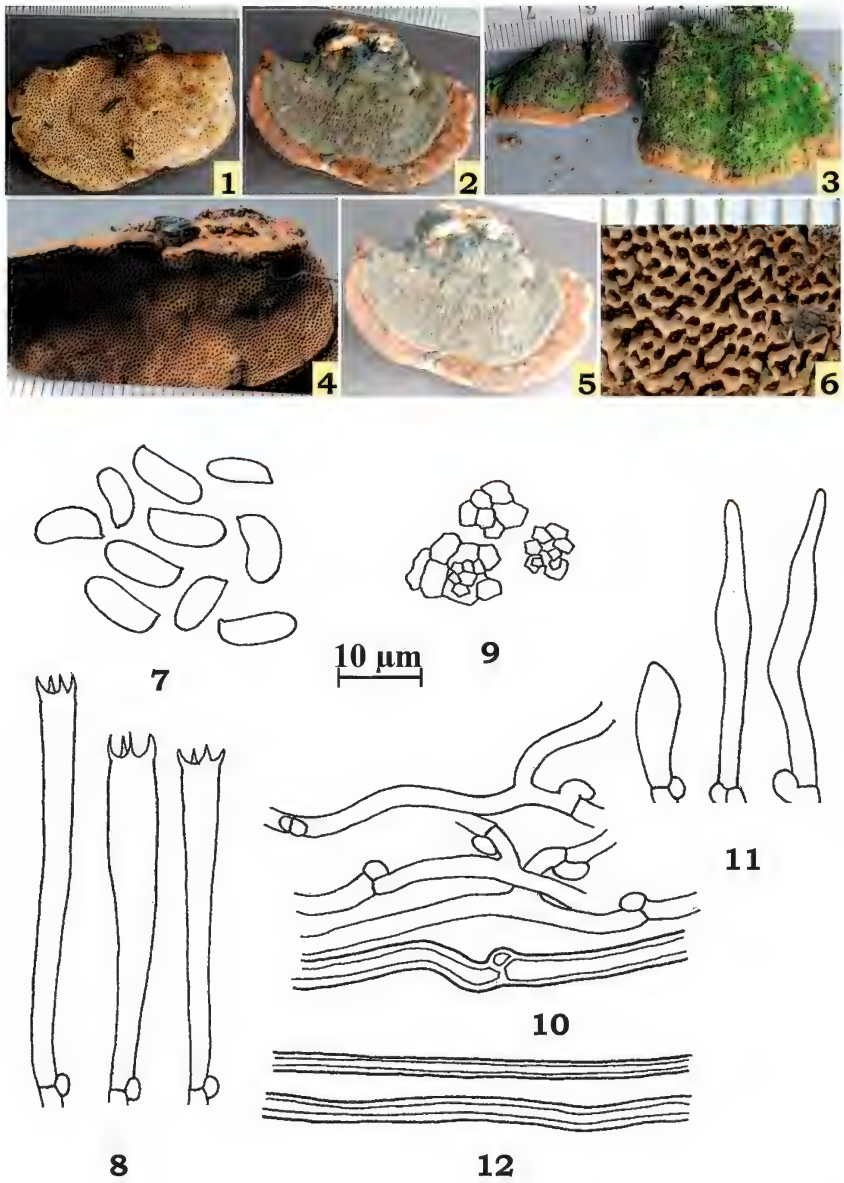

Plate 1. *Datronia ustulatiligna* (holotype). 1. Hymenophore (fresh). 2. Upper part of pileus (fresh). 3. Upper part of pileus covered with mosses (fresh). 4. Hymenophore (dry). 5. Upper part of pileus (dry). 6. Pores (with mm scale). 7. Basidiospores. 8. Basidia. 9. Groups of crystals. 10. Generative hyphae. 11. Cystidioles. 12. Skeletal hyphae.

to dark brown when fresh, do not change much on drying; hymenial surface poroid, light brown to brown when fresh, brown to dark brown on drying; pores angular to daedaleoid, 2–4 per mm; dissepiments thick to thin, entire to lacerate; context ≤2 mm thick, somewhat duplex near base, brown; pore tubes ≤2.0 mm long, brown; scattered groups of crystals observed in trama; margins acute to obtuse, regular to lobed, sterile ≤1 mm, concolorous. Hyphal system dimitic. Generative hyphae ≤3.3 µm wide, branched, septate, clamped, thin–walled in subhymenium and thick–walled in context, subhyaline. Skeletal hyphae ≤3.3 µm wide, rarely branched, aseptate, thick-walled, yellowish brown. Cystidia absent, but cystidioles present in hymenial layer, 16–37 × 4–5.3 µm, fusoid, thin-walled. Basidia 40–49 × 4–6 µm, narrowly clavate, 4-sterigmate, subhyaline, clamped at the base; sterigmata up to 3.3 µm long. Basidiospores 7.3–9.8 × 2.5–4 µm, ellipsoid to subcylindrical to somewhat allantoid, smooth, subhyaline, with oily contents, thin–walled inamyloid, acyanophilous.

Additional specimens examined: **INDIA, Himachal Pradesh, Shimla,** about 4 km from Narkanda towards Rampur, 2 August 2010, on a partly burned gymnospermous stump, Dhingra 5611 (PUN); on a partly burned tree of *P. roxburghii*, Harpreet 5608 (PUN), Harpreet 5613 (PUN); **Sirmaur,** 4 km from Habban towards Rajgarh, 30 August 2011, on a partly burned tree of *Cedrus deodara* (D. Don) G. Don, Harpreet 5612 (PUN).

Remarks– *Datronia scutellata* (Schwein.) Gilb. & Ryvarden differs from our new species by smaller pores (4–5 per mm), shorter and wider basidia (20–30 × 7–10 µm), and association with angiospermous wood.

Acknowledgements

The authors thank Head (Department of Botany, Punjabi University, Patiala) for providing research facilities; Dr. Nils Hallenberg, Professor Emeritus (University of Gothenburg), for peer review and expert comments; and Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.299

Volume 130, pp. 299–304 *January–March 2015*

Three new species of *Xylaria* from China

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Abstract —Three new species, *Xylaria semiglobosa*, *X. sphaerica*, and *X. jiangsuensis*, are described. They were discovered in Yunnan, Hainan, and Jiangsu Provinces in China.

Key words —*Ascomycota,* pyrenomycetous fungi, *Xylariaceae,* taxonomy

Teng (1963) described 51 species and one variety of *Xylaria* from China and Tai (1979) recorded 52 species, one variety, and one form. Ju & Roger (1999) reported 40 species and 1 variety from Taiwan. Since 1979 many new Chinese species and records of *Xylaria* have been published (Li & Li 1994, Xu 1999, Ju & Hsieh 2007, Ma et al. 2011a,b, 2012, 2013, Zhu & Guo 2011, Huang et al. 2014a,b). Three additional new species are now described as follows:

Xylaria semiglobosa G. Huang & L. Guo, **sp. nov.** Figs 1–3

Fungal Name FN570116

Differs from *Xylaria glebulosa* in its larger stromata and smaller ascospores.

Type: China, Yunnan, Mengla, alt. 760 m, on wood, 17.X.2013, G. Huang, L. Guo & W. Li 230 (HMAS 270193, **holotype**).

Etymology: The epithet refers to the stromatal shape.

Stromata gregarious or scattered, semiglobose, on narrow central connective, 3–9 mm high, 4–14 mm diam; surface black, finely reticulately cracked, with inconspicuous perithecial mounds; interior white, becoming hollow at maturity. Perithecia subglobose or ellipsoid, 600–900 µm diam; ostioles papillate. Asci with eight ascospores arranged in a uniseriate or partially biseriate manner, cylindrical, overall 212–237 µm long, 9–16 µm broad, the spore-bearing parts

122–132 µm long, with an apical ring staining blue in Melzer's iodine reagent, urn-shaped, 9–10 µm high, 5–8 µm broad. Ascospores dark brown, unicellular, ellipsoid-inequilateral, sometimes pinched at the one end, smooth, (20–) 22–25(–27) × 6–7(–9) µm, with an oblique germ slit much shorter than the spore length.

Additional specimen examined: **CHINA**, Haina. Diaoluoshan Natural Reserve, on wood, 10.XI.2012, S.H. He 201211101 (HMAS 270192).

Comments: *Xylaria semiglobosa* is similar to *X. glebulosa* (Ces.) Y.M. Ju & J.D. Rogers, which differs in having smaller stromata [(1.5–3) mm high, 1–3 mm diam] and larger ascospores (27–31 × 8–10 µm), frequently with abruptly pinched ends (Ju & Rogers 1999). The new species also resembles *X. fraseri* M.A. Whalley et al., which differs in having whitish scales on the stromatal surface, asci with a shorter apical ring (4.5–5 µm high, 3.5–4 µm broad), and ascospores with a straight germ slit (Whalley et al. 2000). *Xylaria semiglobosa* is close to *X. atroglobosa* Hai X. Ma et al., which differs in its asci with shorter apical rings (4.5–5 µm high, 3.5–5 µm broad) and ascospores with a round hyaline non-cellular appendage up to 6–7 × 3–4 µm long (Ma et al. 2012).

Xylaria sphaerica G. Huang & L. Guo, **sp. nov.** Figs 4–6

Fungal Name FN570117

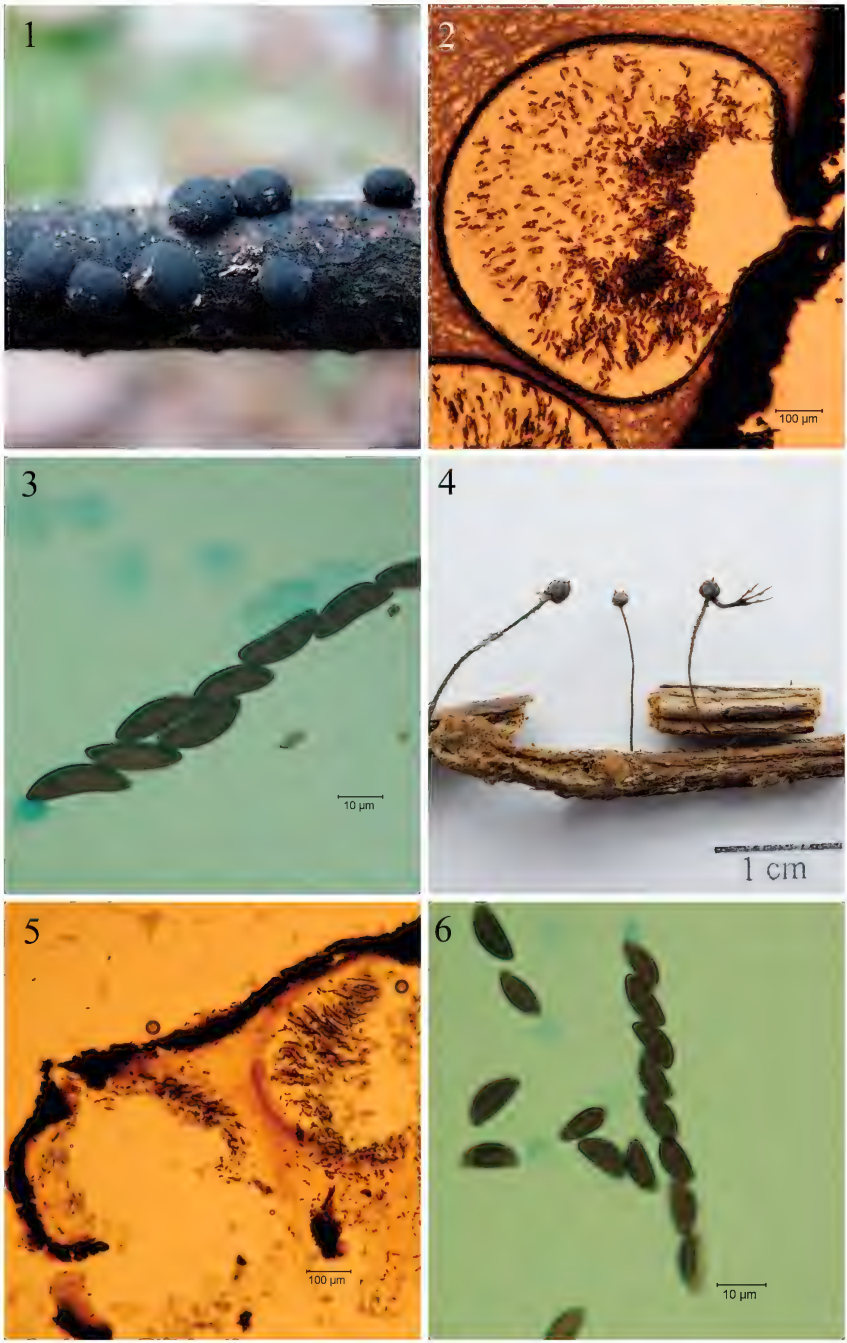
Differs from *Xylaria sicula* f. *major* in its stromata on wood with a small acute sterile apex and long branched protrusion near the base of the head and its slightly larger ascospores.

Type: China, Hainan, Bawangling Natural Reserve, alt. 740 m, on wood, 12.XII.2009, Y.F. Zhu & L. Guo 133 (HMAS 270191, **holotype**).

Etymology: The epithet refers to the stromatal shape.

Stromata with small acute sterile apex and long branches protruding near the base of the head, 1.3–1.5 cm total length, fertile parts spherical or subsphaeroidal, 1–1.5 mm high, 1–2 mm diam; on tomentose hair-like stipes; surface black, smooth, with perithecial mounds; interior white. Perithecia subglobose or ellipsoid, 540–600 µm diam; ostioles papillate. Asci with eight ascospores arranged in uniseriate manner, cylindrical, 118–128 µm total length, 7–12 µm broad, the spore-bearing parts 75–83 µm long, with an apical ring staining blue in Melzer's iodine reagent, hat-shaped, 4–5 µm high, 3–4 µm broad. Ascospores brown, unicellular, ellipsoid–inequilateral, occasionally pinched at the one end, smooth, (10.5–)12–13(–15) × 5–7 µm, with a straight germ slit the length of the spore.

Figs 1–6. ***Xylaria semiglobosa*** (HMAS 270193, holotype). 1. Stromata on wood; 2. Section of stroma; 3. Ascus and ascospores. ***Xylaria sphaerica*** (HMAS 270191, holotype). 4. Stromata on wood; 5. Section of stroma; 6. Asci and ascospores.


1
2
100 µm
3
10 µm
4
1 cm
5
100 µm
6
10 µm

Comments: *Xylaria sphaerica* is similar to *X. sicula* f. *major* Ciccar., which differs in having stromata on fallen leaves and with a long apex, slightly smaller ascospores (9–12 × 3–6 μm), and the absence of protruding branches near the base of the stromatal head (Ciccarone 1946).

Xylaria jiangsuensis Rui S. Wang & L. Guo, **sp. nov.** Figs 7–10

Fungal Name FN570118

Differs from *Xylaria filiformis* in its stromata with branched stipes and its larger and fusiform ascospores.

Type: China, Jiangsu: Baohuashan, on dead leaves, 10.VI.1932, S.C. Teng 977 (HMAS 7263, **holotype**).

Etymology: The epithet refers to Jiangsu Province, where this fungus was first collected.

Stromata filiform, with a sterile apex, 3–7.5 cm total length, 0.5–1 mm diam, fertile parts 3–6 mm long; on hair-like, branched stipes; surface black, smooth, with conspicuous perithecial mounds; interior white. Perithecia globose or subglobose, 450–570 μm diam; ostioles papillate. Asci with eight ascospores arranged in uniseriate manner, cylindrical, the spore-bearing parts 104–110 μm long, 5–7 μm broad, with an apical ring staining blue in Melzer's iodine reagent, inverted hat-shaped, 2–2.5 μm high, 1–1.5 μm broad. Ascospores brownish or brown, unicellular, fusiform-inequilateral, sometimes pinched at the one end, smooth, 16.5–20(–21.5) × 4–5(–6) μm, with straight germ slit the length of the spore.

Comments: *Xylaria jiangsuensis* is similar to *X. filiformis* (Alb. & Schwein.) Fr., which differs in having stromata on unbranched stipes and its ellipsoid-inequilateral and smaller ascospores (11–14 × 5–6 μm; Rogers & Ju 1998).

Acknowledgements

The authors would like to express their deep thanks to Prof. Anthony J.S. Whalley (Liverpool, UK) and Dr. L. Vasilyeva (Vladivostok, Russia) for serving as pre-submission reviewers and to Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review. This study was supported by the foundation of Project of the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-Z-9).

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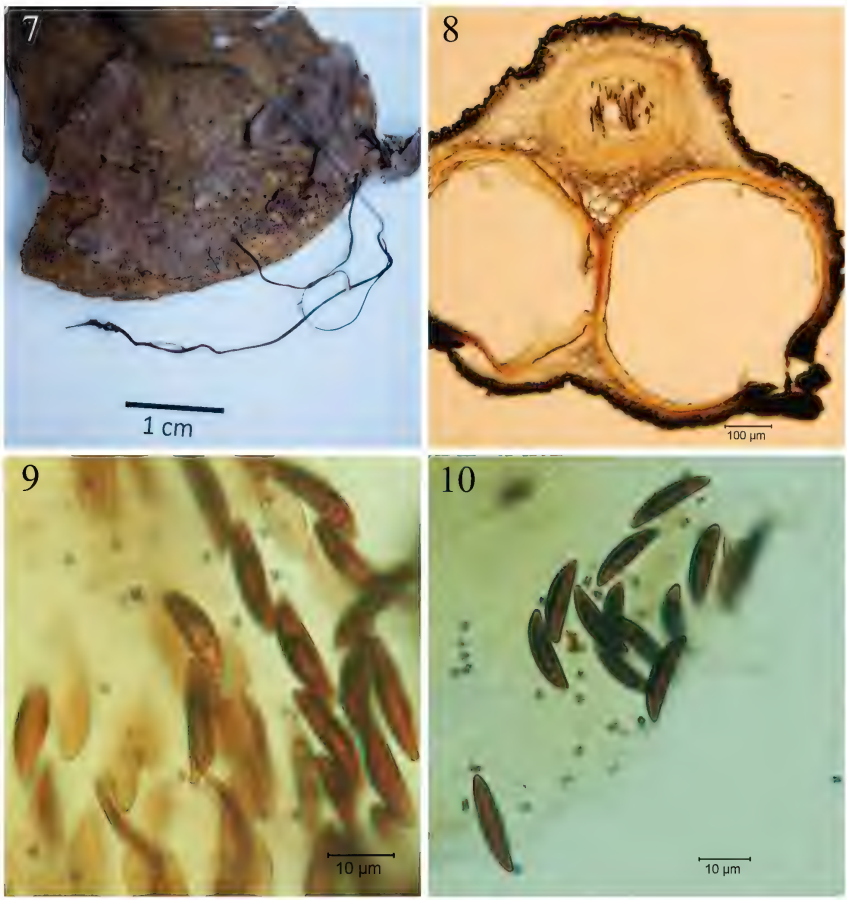

Figs 7–10. ***Xylaria jiangsuensis*** (HMAS 7263, holotype). 7. Stromata on dead leaf; 8. Section of stroma; 9–10. Asci and ascospores.

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ISSN (print) 0093-4666 © 2015 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.305

Volume 130, pp. 305–306 *January–March 2015*

Nomenclatural novelties and typifications proposed in Mycotaxon 130-1

Ambomucor ovalisporus X.Y. Liu & R.Y. Zheng, p. 166
Ambomucor seriatoinflatus var. *longior* X.Y. Liu & R.Y. Zheng, p. 168
Antherospora sukhomlyniae K.G. Savchenko, p. 57
Anungitea guangxiensis J.W. Xia & X.G. Zhang, p. 43
Bertia hainanensis Lar.N. Vassiljeva, H.X. Ma, Chernyshev & S.L. Stephenson, p. 198
Cladonia pseudofissa (Asahina) Ahti, Pino-Bodas & S. Stenroos, p. 98
Coccomyces neolitseae Qing Li & Y.R. Lin, p. 290
Coccomyces prominens Y.F. Xu &Y.R. Lin, p. 74
Datronia ustulatiligna Har. Kaur, G. Kaur & Dhingra, p. 295
Dentipellicula austroafricana Jia J. Chen, L.L. Shen & Y.C. Dai, p. 20
Ellisembia longchiensis J.W. Xia & X.G. Zhang, p. 44
Fistulina subhepatica B.K. Cui & J. Song, p. 53
Hyphoderma hallenbergii Man. Kaur, Avneet P. Singh & Dhingra, p. 223
Lactarius indochrysorrheus K. Das & Verbeken, p. 110
Lactarius olivaceoglutinus K. Das & Verbeken, p. 114
Lactarius pyriodorus K. Das & Verbeken, p. 118
Lactarius yumthangensis K. Das & Verbeken, p. 124
Leiorreuma crassimarginatum Z.F. Jia, p. 248
Lophodermium heterocladum H.L. Gu & Y.R. Lin, p. 192
Perenniporia koreana Y. Jang & J.J. Kim, p. 174
Periconiella liquidambaricola U. Braun, S. Bien & Hönig, p. 254
Periconiella qualeicola Dorn.-Silva & Dianese, p. 257
≡ *Periconiella longispora* Dorn.-Silva & Dianese 2004, nom. illeg. non *P. longispora* Kamal, Suj. Singh & R.P. Singh 1979
Phyllachora hainanensis Na Liu & G. Huang, p. 237
Pseudoplectania lignicola Glejdura, V. Kučera, Lizoň & Kunca, p. 2
Rosellinia brunneola Wei Li bis & L. Guo, p. 233
Sarcoporia longitubulata Vlasák & Spirin, p. 282

Terriera aequabilis Qing Li & Y.R. Lin, p. 28
Tuber xanthomonosporum Qing & Y. Wang, p. 64
Verticicladus hainanensis M.T. Guo & Z.F. Yu, p. 276
Xylaria jiangsuensis Rui S. Wang & L. Guo, p. 302
Xylaria semiglobosa G. Huang & L. Guo, p. 299
Xylaria sphaerica G. Huang & L. Guo, p. 300
Xylaria thailandica Srihanant, Petcharat & Lar.N. Vassiljeva, p. 228
Zasmidium cassines Kharwar, Arch. Singh, Raghv. Singh & Sham. Kumar, p. 242
Zasmidium fabaceicola Kharwar, Arch. Singh, Raghv. Singh & Sham. Kumar, p.244

bad taxonomy

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